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# USSR REPORT

## SPACE BIOLOGY AND AEROSPACE MEDICINE

Vol. 19, No. 4, July-August 1985

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### PROBLEMS OF STUDYING FLIGHT WORK IN SOVIET AVIATION MEDICINE OF THE 1920'S-1930'S

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[Article by V. M. Munipov]

[English abstract from source] This paper describes multifaceted investigations of the flying work by Soviet aviation medicine in the 1920-30s. It examines the early stages of the professional activity that is at present termed ergonomic foundations of the design, manufacture and use of the aircraft. The paper discusses one of the first formulations of the problem of standardization of the human factors.

[Text] At the dawn of its development, Soviet aviation medicine, like many other areas of scientific and practical endeavor, was subject to the expansion of the psychoengineering movement. While propagandizing methods of psychoengineering studies of pilots, S. Ye. Mints was nevertheless the first Soviet aviation physician to indicate, already in the 1920's, that there was a need for physicians to study the work and life of flight personnel, referring to improvement of professional screening methods, deeper approach to optimization of pilot work, systematic investigation of problems of accidents and traumatism, more thorough observation of pilots' health status. In the vast majority of aviation schools, psychophysiological laboratories were established in 1924 under the influence of S. Ye. Mints, while the USSR Administration of the Air Force, by agreement with the Military Medical Administration of the Workers' and Peasants' Red Army, organized a central psychophysiological laboratory. A. A. Sergeyev remarked that "These two steps immediately determined the basic direction of Soviet aviation medicine, which was named psychophysiological, i.e., combining psychological and physiological methods" [15].

Relating the organization of psychophysiological laboratories to a specific stage of development of the preventive direction in Soviet aviation medicine, N. M. Dobrotvorskiy, who headed the central laboratory, called special attention to the fact that with such orientation aviation psychophysiology has much broader tasks than a psychoengineering examination for professional screening purposes. "Approaching the study of aviation from the standpoint of psychophysiology," he wrote in 1924, "we are faced with a specific form of labor that requires psychophysiological characterization. For this reason we must, in the

first place, study the psychophysiology of this labor process itself; in the second place, the subjects who perform this process and in the third place, single out the requirements that must be met by subjects who are the most suitable for performance of these work processes" [18] [sic].

Unlike psychoengineering laboratories with their absolutization of methods of determining the personal traits of an individual by means of tests, in psychophysiological laboratories, the prerequisite for solving problems of professional screening should, according to N. M. Dobrotvorskiy, be a serious examination of pilots under the specific conditions of their work. N. M. Dobrotvorskiy wrote, "Hence it is obvious that to perform the tasks put to us we must analyze in detail both the entire performance of a pilot, as well as himself, using all methods available to us" [8].

The preventive direction of work in psychophysiological laboratories determined the broad spectrum of their tasks to provide optimum conditions for retention and fortification of health, increased work performance of pilots, including working, living, recreation conditions and physical culture.

Striving to investigate comprehensively pilot performance and their working conditions, N. M. Dobrotvorskiy analyzed psychophysiological distinctions of flight work, developed methodological problems of flight training and professional screening of test and observer pilots, validated psychophysiological requirements of flight personnel, etc. His attention was drawn to problems of physical culture in aviation, pilot work standards, diet, professional hazards, etc.

A close link between investigation of pilot performance and equipment (aircraft) was evident already in the first works of the Central Psychophysiological Laboratory. N. M. Dobrotvorskiy attributed much attention to their development: "... We must mention the work of doctor Pereskokov," he wrote, "who initiated studies of an aircraft from the standpoint of its conformity to optimum performance of the pilot's functions and demonstration within the aircraft proper and its lay-out of factors having an inhibitory effect on normal pilot activity" [2].

Inaugurating a new direction of investigations in Soviet aviation medicine, the work of A. Pereskokov were on a par with, if not ahead of, worldwide advances in this field. Proper formulation of the problem, choice of adequate methods and means of solving it, as well as extremely clear ideas about the most effective means of making practical use of investigation results, enabled A. Pereskokov to formulate theses, implementation of which on a large scale began only in our times. In the 1920's, the theses expounded by A. Pereskokov were received with great interest by practical workers (pilots, test pilots and others) [10]. "As for the questions touched upon here (pilot's seat, controls, instruments, etc.)," wrote A. Pereskokov, "specific standards must be developed in this direction on the basis of anatomy and physiology, to conform with aviation equipment specifications, which would enable aircraft designers to develop, along with advances pertaining to flight range, conditions for pilots in which signs of fatigue would appear as late as possible and thereby reach the main goal, that of enabling the pilot to work as productively as possible with the least expenditure of energy" [13].

The work of the Central Psychophysiological Laboratory in the area of optimizing the aircraft cabin and its equipment had some impact on the design of flight vehicles. It is not by chance that, in the document entitled "Bases of Specifications for Military Aircraft" published in 1928, there is a certain list of psychophysiological requirements [9]. N. M. Dobrotvorskiy organically relates this direction of work to the solution of other applied problems. Having made an in-depth and comprehensive analysis of the performance of an aircraft observer, this scientist formulated interrelated recommendations for wiser arrangement of navigational instruments, refinement of organization of pilot work and professional screening [3].

The original ideas for studying flight work, which occurred increasingly often to N. M. Dobrotvorskiy, along with the extensive empirical material that had been accumulated, required systematization and generalization. The scientist was able to do this when he left the Central Psychophysiological Laboratory: "I did not seek a job elsewhere, but started to work at home processing the vast amount of material that I had collected in these years...." [8]. The fact that N. M. Dobrotvorskiy accepted to deliver a course of lectures on the basic problems of aviation medicine for the administrative personnel and engineers at the Air Force Academy imeni N. Ye. Zhukovskiy in 1929-1930 was also instrumental in the performance of that work.

At that time, there was formation of the basic theses of N. M. Dobrotvorskiy concerning the integrated approach to investigation of flight work, which were reflected in the textbook, "Flight Work" [4]. It is also important that elaboration of the theses of the integrated approach to the study of flight work was performed on the basis of an organic link between problems of aviation medicine and engineering problems of designing and developing aircraft: "One-one and a half months later (after N. M. Dobrotvorskiy left the Central Psychophysiological Laboratory.--V. M.), when the basic directions of work were defined, I needed to learn about aircraft blueprints...." [8].

Profound knowledge of the problems of aviation medicine combined with extensive knowledge in the field of aviation and rich practical experience as a psychophysiolgologist and pilot enabled N. M. Dobrotvorskiy to define in a basically new way the structure of the object of investigation of flight work. Having summarized the studies of prior years, N. M. Dobrotvorskiy formulated the objectives of studying flight work as follows: "Investigation of flight work can be divided into several tasks: 1) Investigation of work tools, i.e., the aircraft and its equipment. Investigation of work tools must be pursued from the standpoint of their adaptation to the human material at our disposal, on the one hand, and from the standpoint of selecting human material conforming completely to the specifications imposed on work tools, on the other hand. 2) Investigation of flight work conditions. In studying the conditions of flight work it is not the irrelative effect of these conditions, as considered in hygiene, that is important to us, but the significance of their effect as related to usage conditions (forms of tactical use) in order to determine in advance which people would be most suitable for the performance of different combat assignments, how best to perform these assignments so as to make the fullest possible use of the traits of the living force in our unit [chast']). 3) Investigation of flight work processes. Here, our attention must be concentrated on both the nature of the work processes under study and conditions



that enhance their efficiency from the combat point of view. 4) Investigation of flight personnel. This task has the purpose of solving problems of manning the Air Force with personnel of appropriate quality in the proper shape for flying" [4].

A. A. Sergeyev, describing evolution of the views of N. M. Dobrotvorskiy, calls attention to the fact that it reflects, to some extent, the entire route traveled by Soviet aviation medicine. "Having started with vivid and rather compelling phrases about the need to revise the guidelines for pilot screening and their training on the basis of the Pavlovian teaching on higher nervous activity," writes A. A. Sergeyev, "N. M. Dobrotvorskiy actually took the trail blazed by experimental psychology and, being unable to reconcile these two directions, he went into development of hygienic problems" [15].

In the described thesis of the historian of aviation medicine, it is stated very definitely that N. M. Dobrotvorskiy did not reject either psychology or Pavlovian physiology. It is not actually known whether he was trying to "reconcile these two directions in himself." It appears that for N. M. Dobrotvorskiy the problem was not to "reconcile these two directions," but to define their place in the approach to investigation of flight work that he was validating and which he (and then A. A. Sergeyev also) called "development of hygienic problems." In the 1920's, such concepts as "integrated approach," "ergonomics" and "engineering psychology" did not exist, and for this reason everything that did not pertain to psychology and physiology was called "hygiene." And this is one more manifestation of the pattern, to which V. I. Vernadskiy called attention and according to which "in the history of science the progress of its contemporary development compels us to search and see in its past what previous researchers did not even dream of" [1].

The approach of N. M. Dobrotvorskiy to flight work as an object for investigation having a specific structure, the elements of which perform a functional role in relation to one another and to the structure as a whole, has something in common with the ideas of functional and structural analysis of performance. Without being aware of it himself, N. M. Dobrotvorskiy effected the basic procedures of the structural method to some extent in demonstrating this structure.

In the opinion of N. M. Dobrotvorskiy, investigation of pilots under the specific conditions of their work should be done in both directions at once: man's adaptation to equipment and equipment's adaptation to man. Optimum solutions should be sought where these directions intersect. The formulations of N. M. Dobrotvorskiy are not inferior to many contemporary theses on this score in their thoroughness and completeness. "We believe that requirements can be imposed on man only after the aircraft conforms to the specifications imposed on it by the average man. By no means do we intend to impose the requirement that aircraft must have diverse designs corresponding to the diversity of the groups of people that could be used to work in aircraft--some restrictions must be imposed concerning man, but within these limits the aircraft should meet the demands imposed for the proper use of this average group of people" [4].

A description of the theses developed by N. M. Dobrotvorskiy for an integrated approach to the study and optimization of flight work would be incomplete if we did not dwell specially on the characteristics of one of its elements, namely



the direction of investigations, the content of which is adaptation of equipment to man. In the time of N. M. Dobrotvorskiy, comparatively less attention was given to this direction, and it was even overlooked entirely at times. For this reason, the degree of development of this element of the integrated approach by N. M. Dobrotvorskiy can characterize, in a certain respect, the depth and fullness of his conception as a whole. The approach and concrete results obtained by N. M. Dobrotvorskiy in the course of studying problems in this direction indicate that, in this respect too, his conception holds up to the strictest test of time. For example, with regard to methodology, analysis of the pilot's cabin from the standpoint of the human factor has not lost its relevance to our times. The concrete recommendations made on the basis of this investigation are not generally in contradiction to current ones, although some details, attributable largely to the design of aircraft in those days, are merely of historical interest.

The problem of investigating environmental factors is formulated differently by N. M. Dobrotvorskiy in the structure of the integrated approach to the study of flight work. Their investigation in aviation hygiene of that time, independently of their effect on pilot performance, was in his opinion a mandatory but insufficient factor in the integrated approach. The study of exogenous pilot working conditions within the structure of the integrated approach must be directed, on the one hand, toward definition of the requirements for professional screening and, on the other hand, determination of the means of maximum adaptation of performance to these conditions in cases when it is impossible to change them. Along with such modification of tasks dealing with investigation of environmental factors, hygienic studies retain some independence in the structure of the integrated approach.

Human characteristics had been taken into consideration to some extent or other by engineers in designing aircraft at the start of development of aviation. However, the systematic, scientifically validated approach to their consideration, which was contained in the work of N. M. Dobrotvorskiy, was far ahead of the scientific engineering thought of those times. There are grounds to assume that the sad fact that, expressly at the time of the most intensive development of the integrated approach to the study of pilots under concrete working conditions, i.e., in 1928, N. M. Dobrotvorskiy had to leave the Central Psychophysiological Laboratory is largely related to this circumstance.

In the order to demobilize N. M. Dobrotvorskiy, it was stated that he is relieved from the position he held and for health reasons is discharged on indefinite leave [8]. The scientist himself wrote that "... on 28 February 1928 I parted with the laboratory and became a disabled pensioner at the age of 35 years" [8]. However, the "disabled pensioner" was again admitted to service in 1930 and assigned to the cadre of RKKA [Workers' and Peasants' Red Army] [8] and thus N. M. Dobrotvorskiy became an instructor at the Air Force Academy of the RKKA imeni N. Ye. Zhukovskiy. There is reason to assume that the dismissal of N. M. Dobrotvorskiy from the job of chief of the Central Psychophysiological Laboratory was related not only and, perhaps, not so much to his health as, most probably, to the fact that most aviation physicians did not comprehend the ideas and creative aspirations of the scientist. "The route outlined in keynote papers by N. M. Dobrotvorskiy," remarks A. A. Sergeyev, "did not appeal, for some reasons, to most aviation physicians, and most of them distrusted the achievements of the Central Psychophysiological Laboratory" [15].

It is remarkable that, after the departure of N. M. Dobrotvorskiy, the work of the central and other psychophysiological laboratories was related exclusively and predominantly to traditional problems of aviation medicine. In 1930, when the achievements of these laboratories were summed up, there was discussion exclusively of problems of psychoengineering and psychophysiological screening and medical support of high-altitude and long-term missions [11]. It is equally significant that, having been given the opportunity in 1935 to return to his favorite specialty in a new institute opened at that time, N. M. Dobrotvorskiy conducted a specific series of studies that he had thought about but could not perform in the Central Psychophysiological Laboratory. We refer to studies which can presently, with sufficient grounds, be related to the category of ergonomics (problems of lay-out and equipment of work places in aircraft; onboard comfort as a means of improving combat capacity, etc.). In the 1936 project plan of the Medical Aviation Scientific Research Institute, the topic of "Optimum equipment of aircraft workplaces" was entered for the department of N. M. Dobrotvorskiy. It was stated in the column under the heading, "expected results,": "Navigator's cabin mock-up and physiological specifications for its equipment" [8].

When aviation medicine was only taking its first steps, while differentiation of disciplines dealing with man and his performance was still relatively poorly developed, none of the necessary prerequisites existed for in-depth perception of integrative ideas in these areas of scientific knowledge. The indifference, to some extent, that existed concerning integrative ideas in aviation medicine was not related to the relatively low level of development of aviation equipment. The aviation industry had not yet begun to stimulate integration processes in science.

The conception of N. M. Dobrotvorskiy of an integrated approach to investigation and optimization of flight work acquired some elements of completion [5, 6] during the period of intensive development of the Soviet aircraft industry, when several original aircraft designs were developed that were on a par with the best foreign prototypes in their performance and tactical features. In this regard, the scientist's work, "Onboard Comfort as a Means of Improving Combat Capacity," merits special attention; its very title is indicative of the author's scientific boldness, to some extent. To this day, some specialists believe that comfort and fighting capacity are incompatible concepts. The cited work deals with issues that are presently defined as ergonomic support of designing, developing and operating aircraft. N. M. Dobrotvorskiy wrote: "We construe comfort in an aircraft as referring to all measures related to outfitting the aircraft that provide for convenient work by the crew and preservation of efficiency" [6].

Onboard comfort provisions refer primarily to specific organization of the work place: optimum access, optimum lay-out of controls and instruments. The author calls special attention to the need to optimize pilot performance with respect to reading instruments in the cockpit. N. M. Dobrotvorskiy notes, with regret, that one of the guidelines for such optimization (which was first validated by him and subsequently became an axiom in modern engineering psychology) is still not realized in designing instrument panels in Soviet aircraft cockpits: "In America," writes N. M. Dobrotvorskiy, "they have already implemented the suggestion, first made in our country, concerning warning signals on instruments that automatically attract the pilot's attention to the

instrument. Why should a pilot look at the oil or fuel gage, if all is well with them? It is much simpler if a light goes on, on the control panel, when there is an oil pressure drop or fuel level drops to a specific level, which would alert the pilot to the need to look at the instruments. This simple measure not only relieves the pilot's attention, but enables him to do something else, in particular, help the observer perform combat assignments" [6].

An important element of onboard comfort is to provide specific climate conditions for the crew. As indicated by N. M. Dobrotvorskiy, this can be achieved by optimizing environmental factors in the cockpit and sensible design of work clothes. As noted by N. M. Dobrotvorskiy, it was necessary for aerodynamics specialists testing mock-ups in wind tunnels, to make a more thorough study of the problem of vortexes formed about the fuselage and conditions of air movement within the aircraft in order to improve the arrangement of aircraft cabins of that time, which were far from perfect. "Such studies should provide base data," he stressed, "for work on ventilation of interior cabins of aircraft and air-conditioning, and to provide a comfortable climate" [6].

Comfortable handles, easy to grip, on the control stick, throttle and other controls for the aircraft and its equipment are also not only quite feasible, but mandatory conditions for onboard comfort. N. M. Dobrotvorskiy comments: "The very finish on a control--smooth in some cases and, on the contrary, rough in others, would facilitate the crew's work. Installation on the control stick of a down cuff would enable the pilot to do without cumbersome gloves, in which movement is difficult. Electrical heating of such a cuff would be more reliable than heating the glove, since the cuff would be permanently installed and would not be subject to the negative factors, to which a glove is exposed" [6].

As noted by N. M. Dobrotvorskiy, one could find dozens of places on aircraft of those times, where one could be scratched or bruised, causing decline of crew efficiency. All this, as well as arrangement of hand rails, devices for securing ancillary equipment and many other items may appear, at first glance, as unimportant trivialities; however, comfort cannot be provided in an aircraft without giving the very closest attention to such, seemingly, insignificant "conveniences, all of which together yield, however, a substantial result" [6]. The scientist formulated a thesis which acquired axiomatic relevance in modern ergonomics and design, namely: one cannot provide complete comfort and safety of human work without giving attention to small things. "These simple truths require that the trimmings of an aircraft be just as highly sophisticated as the engineering of our aircraft" [6].

Calling attention to the ever increasing technical level of Soviet aircraft, which made it possible to achieve world records, N. M. Dobrotvorskiy advanced the task of providing the crew with working conditions, under which not only record holders, but simple pilots could achieve the highest results when operating the aircraft, on the basis of consideration of the human factor. "We must now see to it that all installations and equipment in an aircraft are so produced that any average pilot can make full use of the opportunities offered by the aircraft" [6].

In order to solve these problems, it is necessary for scientists and specialists in aviation medicine participate in the work of designers, so that they



could counsel the latter in all questions of aircraft equipment related to the crew's work. In concluding his article, N. M. Dobrotvorskiy states: "A routine task in the joint work of designers, production workers and specialists in aviation medicine to pay strict attention to all this 'trifles', which adapt the aircraft to the requirements of an average person, and furthermore it is a task that will not tolerate procrastination" [6]. At that time, the conception of N. M. Dobrotvorskiy of an integrated approach to investigation and optimization of flight work attracted only a few enthusiasts of aviation medicine. They include the aviation physician, S. P. Rozenberg who was attached to the Central Psychophysiological Laboratory of the Air Force in 1924 and, from 1925 one, worked in the psychophysiological laboratory of the military pilot school in Odessa. Developing the ideas of N. M. Dobrotvorskiy for standardized layout of instruments on the instrument panel, S. P. Rozenberg made the first attempt at validating the set of psychophysiological requirements for standardization of the instrument panel and instruments themselves.

One should rate quite highly the work done by S. P. Rozenberg at a time when there was an acute shortage of psychophysiological data required to work on standardization of aircraft instrument panels and without any positive knowhow in this matter. While some of the specific recommendations he offered did not withstand the test of time, formulation of the problem and the general approach to its solution have not lost their relevance to this day. S. P. Rozenberg is to be credited for the fact that he was the first to implement rather systematically the performance approach to the problem of standardizing the aircraft instrument panel. This approach was implemented on an empirical, rather than conceptual level. Nevertheless, the approach of S. P. Rozenberg properly grasped the general idea and outlined the strategy for optimizing and standardizing aircraft instrument panels on the basis of studying pilot performance [14].

Being ahead of his time in formulating a number of problems of psychophysiology of flight work, S. P. Rozenberg, like N. M. Dobrotvorskiy, found no support in his contemporary aviation medicine. "Having made a brilliant study of pilot work both in theory and practice, he (S. P. Rozenberg--V. M.) tried to rationalize it by psychophysiological methods, but the absence of a school prevented him for working more in-depth and prompted obvious wavering of subject matter" [15].

The conception of an integrated approach to the study and optimization of flight work being developed by N. M. Dobrotvorskiy had some impact on the work of N. V. Zimkin and N. A. Eppe, who were among the first in the world to conduct engineering-psychological studies of aircraft instruments. N. V. Zimkin stated plainly that his research was a continuation of the work of N. M. Dobrotvorskiy, with whom he had conducted experimental psychological studies of pilots in 1921 in the clinic of V. M. Bekhterev. The experimental work of N. V. Zimkin and A. M. Zimkina was related essentially to psychophysiological evaluation of instrument dials as related to illumination conditions.

The work of N. V. Zimkin [7] and N. A. Eppe [16] constitutes an example of brilliant engineering psychological investigation of aviation instruments for their time and, in a number of respects, for our times as well, with respect to formulation of the problem, experimental methods, system and comprehensiveness of the research that was undertaken, as well as results achieved.

To sum up the problems discussed in the area of studying flight work in Soviet aviation medicine of the 1920's and 1930's, it should be noted that it generated and developed the ideas of an integrated approach to investigation, as well as a cycle of studies based on this approach of conditions and optimization of flight work, including development of psychophysiological and hygienic specifications for aircraft and aircraft equipment design. This is all the more important since they were subsequently (particularly in our times) submitted to fundamental development. The work of N. M. Dobrotvorskiy and a few of his proponents is among those that, already in our time, V. V. Parin and I. M. Khazen related to one of the most promising trends of aviation medicine. They wrote: "Aviation medicine not only had to be in step with aviation engineering, but to anticipate the routes of its further development. And this exceptional distinction, which is inherent in all its stages, prevailed in close collaboration of biology, engineering and medicine" [12].

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SOME ASPECTS OF DETERMINING HUMAN PHYSICAL WORK CAPACITY UNDER HYPERBARIC CONDITIONS

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[English abstract from source] Physical work capacity of man during real and simulated dives is discussed in view of the reported data. The parameter is known to decrease as the breathing gas density and immersion depth increase. The factors limiting the work capacity growth are as follows: greater respiration resistance, higher energy cost of ventilation, CO<sub>2</sub> retention, dyspnea, adverse circulation changes. During exposure hyperbaric bradycardia occurs both at rest and upon a work load. This precludes prediction of physical work capacity on the basis of the heart rate alone as a parameter used in a normobaric environment. The paper contains tabulated data on the work capacity of divers at various depths obtained by different authors.

[Text] The most important achievement of the last decade in the area of underwater physiology is the experimental confirmation of human capacity to spend a long time at significant depths. Exploration of marine depths requires investigation of the effect of hyperbaric conditions on man, a search for the means of preserving and improving work capacity at high ambient pressure.

Much attention is given in theoretical and clinical medicine to the study of physical work capacity. In spite of the fact that a different content is placed in the concept of physical work capacity in different branches of medicine, there is still a general definition of this concept as man's potential capacity to manifest a maximum physical exertion during work [12, 17, 26, 34, 38].

As we know, human physical work capacity depends on individual distinctions, chiefly the functional state of an individual's cardiorespiratory system. While special significance is attributed to the cardiovascular system at normal atmospheric pressure, in the presence of high pressure this applies to the respiratory system. The specifics of a hyperbaric environment consist of the fact that expressly respiration could limit an increase in work capacity, whereas some reserve is still left in the circulatory system.

The limiting role of the respiratory system is related primarily to increase in density of the atmosphere, in which an aquanaut lives and works.\* High ambient density results in an increase in resistance to respiration, which depends primarily on the nature of gas flow in airways. While gas flow is mostly streamline during quiet breathing, during physical labor even at normal pressure the increase in ventilation leads to formation of turbulent flow. An increase in ambient density has the same effect; already at a pressure of 5-6 kg/cm<sup>2</sup>, virtually the entire gas flow becomes turbulent [45, 51].

As we know, an increase in resistance to respiration leads to slowing of inspiratory and, particularly, expiratory flow [62, 84]. The decline of peak flow velocities under hyperbaric conditions plays an appreciable role in limiting growth of ventilation during forced breathing associated with physical labor [70, 81]. The limitation of peak flow velocities has the most appreciable effect at heavy physical loads or during the maximum breathing capacity (MBC) test on the basis of which determination is made of reserve capacity of the respiratory system. Theoretically, the decline of MBC at high pressure should be inversely proportionate to the square root of gas environment density [47, 54, 60, 64, 67]. But in reality, MBC at high ambient pressure is somewhat higher than the theoretically calculated levels [56, 60, 70]. It is assumed that this occurs due to adaptive reactions such as increase in activity of respiratory motoneurons and consequent intensification of contractions of respiratory muscles [20, 29, 33, 69]. However, this mechanism of maintaining physical work capacity can prevail for only a brief time, since maximum exertion of respiratory muscles leads to development of fatigue.

In addition, growth of pulmonary ventilation during physical exercise diminishes for another reason. At high velocities of gas flow and with significant expiratory exertion, intrathoracic pressure becomes higher than pressure in the bronchi. This leads to collapse of fine bronchi, and further efforts to overcome the increased resistance to expiration are no longer effective. It is believed that this mechanism lowers ventilation by about 20% [49], whereas the maximum capacity of pulmonary ventilation may constitute about 60-80% of MBC according to the results of experimental studies [50, 73].

The increasing energy cost of respiratory muscle function, due to increased resistance, as well as the sensation of dyspnea, may become factors that limit work capacity under hyperbaric conditions.

At normal atmospheric pressure, the output of respiratory muscles at rest constitutes  $0.23 \pm 0.5$  kg-m/min [16]; it is 6-12 kg-m with ventilation of 60 l/min and 250 kg-m during maximum work at MBC of up to 200 l/min [36, 61]. Under hyperbaric conditions, when resistance to respiration is increased, the work of respiratory muscles must increase with increase in atmospheric density.

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\*This survey deals with the effects of high atmospheric pressure mainly in the presence of normal partial oxygen tension. The effects of hyperoxia will not be discussed here, although several works are cited where compressed air was used. Nor will an explanation be offered here concerning questions about the effects of ambient temperature and humidity.

In actual measurements of respiratory muscle function at high ambient density, an increase is not necessarily demonstrable at rest. This can be attributed to relatively low level of ventilation and compensatory change to slower and deeper respiration [3]. As we know, such a respiration pattern provides for optimum ventilation conditions, where a minimal amount of energy is expended on the function of respiratory muscles. Probably it is this optimization of respiration pattern that explains the contradictory results of experimental studies: decline of power of respiratory muscle function from 8 to 6 W with maximum physical load and from 28 to 18 W with MBC at a pressure of 6 kG/cm<sup>2</sup>, as compared to values at a pressure of 1.3 kG/cm<sup>2</sup>.

Direct measurement of respiratory muscle function involving measurement of pressure in the thoracic cavity (or esophagus) is often difficult under hyperbaric conditions. To assess respiratory muscle function, calculation was made of oxygen cost per liter ventilation. It constitutes 0.5-1 ml O<sub>2</sub> on "earth" at rest. If, however, ventilation reaches a maximum, at both MBC or close values, all of the incoming oxygen is spent on respiration [15]. According to estimates, the same effect at rest is obtained with 30-fold (true, this is hypothetical as yet) increase in density of the helium and oxygen environment, which is equivalent to a depth of 2000 m.

Actual determination of oxygen cost per liter ventilation under hyperbaric conditions revealed that it changes in accordance with ambient density, but the measurements obtained by different authors do not always coincide. For example, 5-fold increase in density raised the energy cost per liter ventilation by 1.53 times; 7-fold increase, by 2.25 times [23]. When the inert component of the respiratory mixture was sulfur hexafluoride (SF<sub>6</sub> gas) and mixture density at normal atmospheric pressure was increased by about 4 times, the energy cost of ventilation was also 3.6-4.5-fold increased [3].

Can the increase in oxygen cost of ventilation affect total oxygen uptake ( $\dot{V}O_2$ )? Theoretically, it can, particularly when functioning at critical power; however, the accuracy of measuring  $\dot{V}O_2$  plays a large part. It is common knowledge that respiratory muscles take up no more than 2-3% of total  $\dot{V}O_2$ , even during heavy labor. Moreover, as indicated above, with increase in ambient density one observes changes in respiratory pattern in the direction of economical function of respiratory muscles. For this reason,  $\dot{V}O_2$  may differ from the control level up to a certain limit for both density and functional power.

The data on  $\dot{V}O_2$  readings at different depths cited in different studies are contradictory.

Function at 1200 kG/min elicited a 28% increase in  $\dot{V}O_2$ , 61% increase in oxygen demand and 137% increase in oxygen debt at a pressure of 5 kG/cm<sup>2</sup> in an atmosphere of compressed air. There was also an increase in time of elimination of oxygen debt, as compared to normal atmospheric pressure. These parameters increased even more at a pressure of 7 kG/cm<sup>2</sup>:  $\dot{V}O_2$  by 34%, oxygen demand by 67% and oxygen debt by 163% [23, 25]. Such significant rise in oxygen cost of work can apparently not be attributed solely to increase in energy expended for respiration; rather, one could think of decrease in economy of useful work under the influence of stress factors.



Thus far, measurement of  $\dot{V}O_2$  during physical labor at high pressure of helium-oxygen environment has not yielded unequivocal results. According to some authors, this parameter was lower than during analogous work on "earth" [68], and from the information of others it did not change appreciably [77] or rose [52, 71, 83]. Thus, the question of effect of high pressure on energy cost of exercise cannot be considered answered as yet.

Since physical labor, no matter where it is performed, involves rise in gas exchange, it is necessary to examine the correlations between ventilation reactions and process of  $CO_2$  formation, which progresses with increase in exercise load. At normal atmospheric pressure, an increasing load leads to increase in ventilation, which permits removal of excessive  $CO_2$  entering the lungs. In the case of a moderate physical load,  $p_A CO_2$  and, accordingly,  $p_a CO_2$  change insignificantly; heavy and prolonged work elicits hyperventilation with excessive removal of  $CO_2$  and development of respiratory alkalosis.

According to a number of authors [53, 65, 75], at high pressure and corresponding increase in density, during work of varying intensity there was an increase in  $CO_2$  content of alveolar air, from 46 to 52-56 mm Hg and in some cases even more. It was previously reported that  $P_A CO_2$  rises to 70 mm Hg and there are signs of respiratory acidosis (blood pH was 7.21) in divers working at up to 900 kG/min and nitrogen-oxygen atmosphere pressure of 5 kG/cm<sup>2</sup> [23, 37]. It was also reported that  $P_A CO_2$  rose to 76 mm Hg in a diver engaged in heavy labor at a pressure of 4 kG/cm<sup>2</sup>; however, it was noted that there was an extremely mild respiratory reaction to the work load in this case [65].

What causes  $CO_2$  retention when working under hyperbaric conditions? Studies of Soviet and foreign physiologists revealed that, at high density, there is considerably less marked increase in ventilation during physical work than when performing the same work on "earth" [5, 40, 78, 83]. Accordingly, there is a decline in rise of alveolar ventilation. Consequently, the conditions for removal of produced  $CO_2$  become considerably worse than at normal atmospheric pressure.

Diminished sensitivity of chemoreceptors and the central element of the system of controlling respiration to the hypercapnic stimulus,  $CO_2$ , is another cause of retention of the latter in the body. Such a decline in sensitivity to  $CO_2$  was demonstrated in divers, even when breathing quietly [58, 74].

It is believed that this could be related to increase in tidal and vital lung volume, as well as depth of respiration. A special survey demonstrated some correlation between frequency, depth of respiration and low reaction to  $CO_2$  [73]. Perhaps, aquanauts and divers acquire adaptation to elevated  $p_A CO_2$  during their professional work with regular submersions [74]. At the same time, it can be lost after 3 months at normal atmospheric pressure [53]. Some authors [52] even suggest that aquanauts be screened on the basis of individual reactions to  $CO_2$ .

The results of studying respiratory center reactions under hyperbaric conditions revealed that the threshold of the ventilation reaction to  $CO_2$  is about 20% lower in experienced divers [44]; consequently, their  $p_A CO_2$  during work may be higher than in individuals engaged in other occupations.



Inadequate pulmonary ventilation and its increased energy cost under hyperbaric conditions may be manifested in the form of dyspnea. The latter is defined as a sensation of shortage of inhaled gas, difficult respiration, "short inspiration" and inability to "catch one's breath" [49, 72]. The degree or severity of dyspnea cannot be measured quantitatively, and it is determined only by sensations. One aquanaut working underwater defined the sense of dyspnea as "terror, the equal of which I never experienced before" [59].

Increased work of respiratory muscles,  $\text{CO}_2$  retention, high ventilation and metabolic acidosis are considered to be factors leading to dyspnea. These factors may occur during maximum work at moderate depths. At pressures of 43-59  $\text{kG/cm}^2$ , dyspnea limits work capacity, even without appreciable changes in the cardio-respiratory system [48]. At depths in excess of 500 m, dyspnea has also been observed at rest: when eating (chewing), drinking, talking, sleeping [72]. The mechanisms of such "hyperbaric dyspnea" are not quite clear. It is assumed that pulmonary ventilation becomes inadequate during work not only as a result of restriction of expiratory flow by compression of airways, but due to decrease in inspiratory exertion due to fatigue of respiratory muscles. Such insufficiency of inspiration can be compared to analogous signs in patients with bronchial asthma or pulmonary emphysema [78].

It is assumed that inspiratory neurons of the respiratory centers, the activity of which shows reflex increase with increase in resistance to respiration, are involved in the origin of sensations of dyspnea [79]. Moreover, at great depths, one cannot rule out the adverse effect as well of the nervous syndrome of high pressure, which perhaps affects myoneural structures of the respiratory system [49]. Impairment of ventilation-perfusion relations and difficult intrapulmonary diffusion are believed to be causes of dyspnea [72], although there is still no direct evidence of this.

According to some data, additional measures (for example, application of mildly positive pressure to the mouth) alleviated dyspnea and increased work capacity of aquanauts [49]. Nevertheless, dyspnea during physical labor at great depths is still a terrible and little-studied symptom, which limits not only work capacity but, perhaps, depth of submersion as well.

Aquanaut work capacity under hyperbaric conditions depends, of course, not only on respiratory system function, but to a significant extent on the state and conditioning of the circulatory system. It is believed that high ambient pressure has an indirect effect on the cardiovascular system, through the respiratory system [30]. At the same time, some researchers also see a direct link between pressure and cardiovascular system function: hyperbaric factors have a direct effect on the heart's conduction system and on the parasympathetic system [50].

There are the most frequent reports of change in heart rate (HR) [35, 74, 76, 83]. Submersion at small and average depths usually causes slowing of HR [9, 10, 24, 28, 31, 66], which could be called hyperbaric bradycardia. True, in some cases there was also quickening of the pulse during and after compression [40]. There is also a less marked HR increment when working under hyperbaric conditions than at sea level. For example, during maximum work at a pressure of 31  $\text{kG/cm}^2$ , mean HR constituted 162/min, whereas on "earth," during similar work, it was 179/min [68].

In the opinion of some authors, hyperbaric bradycardia is a phenomenon related to ambient hydrostatic pressure, although no definite relationship was demonstrable between extent of HR decline and pressure. A special investigation was conducted to determine the role of the sympathetic and parasympathetic nervous system in HR control during work under hyperbaric conditions [50]. Administration of cholinergic and adrenergic agents (propranolol and atropine) revealed that bradycardia elicited by high density and partial nitrogen pressure is attributable to inhibition of the sympathetic nervous system, whereas hyperoxia affects the parasympathetic system. The authors conclude that the observed cardiodepressing effect can be relevant to limiting work intensity under hyperbaric conditions.

A decline of HR also leads to change in circulation volume (CV). When submerging to depths of 12-30 m, this parameter dropped somewhat [19]; at a pressure of 6 kG/cm<sup>2</sup>, the CV decline constituted 1 l/min due to decline of both HR and stroke volume [35, 76]. A decline of stroke volume and CV in a helium-nitrogen-oxygen atmosphere was also recorded at a pressure of 11 kG/cm<sup>2</sup> [28]. At the same time, there are data to the effect that CV remained in the base range when pressure rose to 26 kG/cm<sup>2</sup> [83], and that this parameter rose by 10-20% with 5-fold increase in density [30]. In these cases, there was an increase in stroke volume during work of moderate and maximum intensity at a pressure of 18.6 kG/cm<sup>2</sup> in a helium and oxygen environment [46]. It is believed that stroke volume reaches maximum values sooner under hyperbaric conditions than on "earth" with analogous work [30].

Electrocardiograms, which were recorded during many submersions, showed some changes indicative of the effect of hyperbaric conditions on heart function. Even at small depths, particularly during work, there were changes in automatism function and conduction in the myocardium [8], decrease in wave voltage, extension of P-Q interval, sometimes to a total block, decrease in amplitude of T wave, depression of S-T interval and occasional sinus arrhythmia [13, 22, 82]. All these changes are indicative of an overload on the myocardium, impairment of its metabolism and coronary insufficiency [13, 14, 22, 28]. As a rule, the parameters of cardiac function revert to normal after decompression.

As for the other hemodynamic parameters, we should mention, first of all, variability of the obtained findings: elevation of diastolic pressure when submerging and working at a depth of 30-60 m in an atmosphere of compressed air [11, 27, 32], drop of systolic and pulse pressure [32, 63, 76], as well as in blood flow rate [32], increased resistance in the pulmonary circulation [1].

In spite of the submitted data indicative of adverse changes in some hemodynamic parameters under the effect of a hyperbaric environment, we still must concur with the opinion of some authors to the effect that circulatory function is not a factor that limits appreciably the depth of submersion and work capacity of aquanauts [57].

One of the main tasks of hyperbaric physiology is predetermination of work capacity of an aquanaut or diver at different ambient pressures. In sports medicine, one generally predicts maximum work capacity by the PWC<sub>170</sub> test, using, for example, the following formula:

$$PWC_{170} = N_1 + (N_2 - N_1) \frac{170 - f_1}{f_2 - f_1}$$

where  $N_1$  and  $N_2$  are loads on bicycle ergometer,  $f_1$  and  $f_2$  are the corresponding pulse rates. As applied to hyperbaric conditions, this formula cannot apparently be used for the following reasons. Performance of any work under hyperbaric conditions, as well as on "earth," is associated with acceleration of pulse. However, while there is a linear relationship between heart rate and  $\dot{V}O_2$ , which is a direct indicator of work load, within a certain range, at normal atmospheric pressure, this function is apparently impaired at elevated pressure due to hyperbaric bradycardia: pulse rate may rise to a lesser extent than with analogous loads on "earth." Then the values calculated using the above formula could be higher than the actual maximum work capacity of an aquanaut. The classification of intensity of work as a function of pulse rate developed for normal atmospheric pressure must, in the opinion of most specialists, be corrected when assessing work in a hyperbaric environment [13, 23]. Prediction of work capacity under hyperbaric conditions should probably be based on other methods.

For this purpose, one can use the data on the limiting role of respiration. Considering the consistent decline of MBC with increase in density of the gas environment, one can estimate the level of pulmonary ventilation and, consequently, intensity of work that an individual can perform at a given depth. Such estimates lead to the conclusion that, for moderate loads (up to 750 kg-m/min), 9-fold increase in ambient density can be considered the maximum and 6-fold, submaximum (900-12,000 kg-m/min) [4, 41, 80]. Other estimates based on maximum expiratory flow indicate that it should not exceed 3 l/s at a density of 4-7 g/l. But even under such conditions, maximum work capacity of an aquanaut is limited, and it is impossible at a density of 8 g/l or more [64]. At the same time, the results of studies of recent years revealed that such extrapolations cannot be considered a reliable method of predicting work capacity at significant depths. Direct determination of maximum oxygen uptake (MOU) is still the most reliable.

MOU can be considered the integral parameter characterizing adequacy of respiratory and cardiovascular system function and level of physical work capacity.

At normal atmospheric pressure, MOU is 3.5-4 l/min in conditioned men 20-30 years of age and up to 5 l/min in athletes [2]. MOU has been relatively seldom tested at high pressure, probably due to the danger of decompression disorders that could be provoked by heavy physical loads [6, 73] and of dyspnea. At low depths (30-60 m), MOU reached the same values with maximum loads as on "earth" [7, 18, 73] or diminished [55]. MOU at depths of 60-80 m usually also failed to change in divers with extensive experience in underwater work, constituting a mean of 3.75 l/min [23]. No changes in MOU were demonstrable as well with maximum load at a "depth" of 186 m [46]. However, MOU of aquanauts decreased from 3.11 to 2.71 l/min at a pressure of 31 kg/cm<sup>2</sup> in a helium and oxygen atmosphere [68]. A substantial difference in MOU was also observed at pressures of 1.6 and 43.6 kg/cm<sup>2</sup> during real dives; MOU constituted 2.9-3.0 and 1.8-2.4 l/min, respectively [48].

In the opinion of these authors, MOU during real submersions is "in striking contrast to extrapolations based on observations of divers in dry chambers, where they breathed with gases of comparable or higher density at lower depths...."



# Work load of aquanauts at different pressures of helium-oxygen atmosphere

Pressure kG/cm <sup>2</sup>	Work load, kg-m/min	Refer- ence
31.0	1230	[68]
31.3*	735*	[71]
33.6	1200	[56]
46.0	300	[66]
46.7	1080	[72]
50.0	450	[42]
51	670	[41]
65.6**	810	[72]

\*Maximum pressure and maximum work performed under these conditions are given for several pressure levels.

\*\*5 or 10% nitrogen was added to the helium and oxygen atmosphere

Determination of MOU is inseparable from quantitative description of maximum power that man can develop at different depths. Information about maximum loads with which an aquanaut can cope at high pressure is contradictory. In 1960-1970, it was believed that a diver could perform work of 220-850 kg-m/min at depths of up to 200 m [21, 39, 53, 63]. In the last decade, when diving depths increased significantly, our conceptions of aquanaut work capacity were somewhat expanded.

As can be seen from the Table, even when pressure was raised to 66 kG/cm<sup>2</sup>, it was possible to perform work of 810 kg-m/min, which is 91% of the maximum, for a brief time (6 min).

Nevertheless, in the opinion of most researchers [5, 72, 78], maximum work capacity is diminished at high ambient pressure. Intensity and duration of work are already limited at depths of 490-610 m, although it is assumed that man could use a helium and oxygen breathing mixture at rest to depths of 1500 m [57].

A particular decline of work capacity was observed in aquanauts who worked directly in water, rather than with simulation of immersion. At a depth of 436 m, maximum work capacity was 30% lower [48] and at 560 m 60-70% lower [79] than at a depth of 6 m.

Thus, in the opinion of most prominent physiologists, human work capacity is more or less diminished under hyperbaric conditions. True, the extent of this decline can be determined only approximately in quantitative terms, since indirect methods of measuring work capacity are unsound as yet, whereas direct ones, according to maximum oxygen uptake, are difficult to use in most cases. However, submersions made in recent years warrant the belief that moderate loads were tolerable at the achieved "depths" (656 m) [42]. Further increase in pressure and density of the gas environment inevitably leads to limitation of work capacity.

The increased load on the respiratory function and diminished functional reserves of the cardiorespiratory system constitute the main cause of such limitation. The leading role in limiting work capacity is attributed to different factors: high resistance to gas flow in the airways [43, 57], expiratory collapse of bronchi [51], hypercapnia [71], dyspnea [69]. Unfortunately, the question of the role of fatigue of respiratory muscles in a dense environment has been studied very little [70]; nor is it clear how hyperoxia and adaptation to hyperbaric conditions affect work capacity. One should also take into consideration the possibility of impaired work capacity due to the effect of

hyperbaric factors on central nervous system function. Aside from the stressor effect of deep-sea diving, we should include here the specific effect of high partial pressure of neutral gas. One cannot fail to take into consideration also the effect of heat-transfer conditions, especially during real dives.

Apparently, future investigations of the effects of the entire set of hyperbaric factors will help preserve aquanauts' work capacity at an adequate level.

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EXPERIMENTAL AND GENERAL THEORETICAL RESEARCH

UDC: 629.78:612.014.477-063-08

INVESTIGATION OF FACTORS DETERMINING PILOT'S GEOCENTRIC ORIENTATION

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 8 May 84) pp 19-23

[Article by V. V. Lapa, Ye. Ye. Bukalov and N. A. Lemeshchenko]

[English abstract from source] The use of a rational (geocentric method of orientation in flight is determined by the specific content of the conceptual model that develops in the course of flying experience and by the display of the spatial position of perceptive signs of the geocentric system of coordinates. The importance of these factors is confirmed by erroneous decisions made by operators with no flying experience (40%) when they estimated the spatial position as well as by a shorter time and a lower number of errors made in assessing the spatial position when the display presented signs of the geocentric system of coordinates.

[Text] Flight safety and efficiency in modern aircraft depend significantly on the pilot's capacity to orient himself in the space around him correctly and continuously. In this respect, flights made beyond visibility of natural ground-based reference points are the most difficult. In this case, the pilot is dealing with artificial means of displaying the spatial position of his aircraft. These means help, to some extent or other, construct and maintain a mental picture of the aircraft's spatial position and motion, the so-called image of the flight [1]. The question of the meaningful aspect of flight image, more precisely its basic component--image of spatial position--is drawing the increasing attention of specialists in aviation medicine. The results of experimental studies revealed that the indicators used during flight must provide for the formation and function of an image of spatial position reflecting spatial relations in a geocentric system of coordinates,\* since the pilot in flight views his (the aircraft's) displacement in relation to the stationary ground. It is expressly in this case that the pilot's

\*This refers to use of some element in the environment as the reference point for orientation. In addition to the geocentric system, it is possible to use an internal system of coordinates, where man relates objects in the environment to the main orientations of his own body--the egocentric method of orientation.

efficient and reliable spatial orientation is assured [4, 5]. However, we still do not have a complete idea about factors and conditions that determine the geocentric method of pilot orientation.

On the basis of conceptions of the mechanisms of mental regulation of pilot actions in flight when the ground and natural reference points are not visible, which were developed by V. A. Ponomarenko and N. D. Zavalova [7], we assumed that use by the pilot of the geocentric orientation method is determined, in the first place, by the specificity of the content of the flight image (conceptual model) formed in the course of professional work and, in the second place, by the degree of conformity of the information model to the content of this image. One of the means of effecting such conformity is to display in the information model the perceptive signs (elements) of the geocentric system of coordinates.

Experimental investigation of the role of the factors singled out for use of the geocentric method of orientation in flight was the subject of this study.

### Methods

Tests were performed with pilots and operators without flying experience in order to determine the role of the content of the image of spatial position formed in the course of flying. Real flights were made in an aircraft using a laser landing system [2].\* Determination was made of efficiency and reliability of the subjects' assessment of spatial position after partial disorientation. The latter was produced by the following method. The instructor led the aircraft away from the given landing trajectory (with a blind over the instrument panel and glass on the dome light). After opening the blind, the pilot's or operator's (they sat alternately in the aircraft cockpit, in the copilot's seat) task was to assess the spatial position of the aircraft and report it to the instructor. In the tests, we simulated deviations of the aircraft from the landing path with regard to three parameters, heading, glide path and bank.

We examined the role of content and structure of the information model (IM) in the laboratory. Variants of information models (Figure 1) were presented to the pilot on a tachistoscope; they differed from one another in number of perceptual signs of external space (lines of artificial horizon, linear perspective, outlines of runway, silhouette of aircraft). In variant A, which served as the baseline, only the landing symbol of the laser landing system was displayed. In the other variants (B, C, D and E), the above elements were included, in addition to the symbol. Slides depicted the forms of IM with deviations of the aircraft from the specified trajectory in heading, glide path and bank. The pilot's task was to assess the aircraft's spatial position

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\*In this system, the spatial position of the aircraft is induced by three extended reference points (laser beams) visible in space, one of which designates direction (heading) of landing and the two others, the specified trajectory (glide path) of descent. Beams projected on the plane create a landing symbol, the shape of which determines the aircraft's position on the landing trajectory.

on the basis of the presented picture. The slides were presented in random order. Presentation time was not limited.

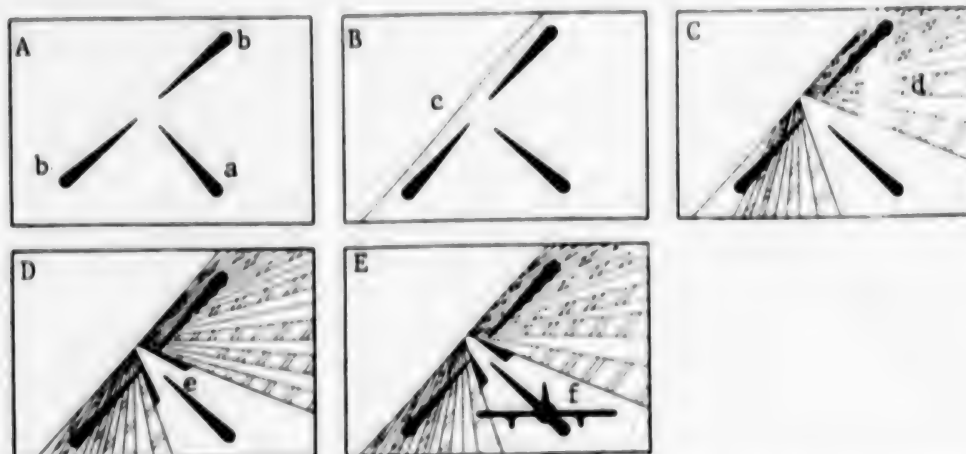


Figure 1. Types of IM. Shape of landing symbol (A-E) corresponds to aircraft's position as to heading, glide path, with right bank

- |                            |                           |
|----------------------------|---------------------------|
| a) heading beam            | d) linear perspective     |
| b) glide path beams        | e) outline of runway      |
| c) artificial horizon line | f) silhouette of aircraft |

The following were used as criteria to evaluate efficiency and reliability of spatial orientation: latency period of restoration of orientation (from the time the blind was opened to the start of a verbal response); erroneous decisions in assessing situation; data in verbal report and from interrogation of subjects.

A total of 10 pilots and 10 operators participated in this study. A total of 150 situations were simulated in actual flight and 650 in the laboratory.

## Results and Discussion

### Actual Flight

Let us refer to the results obtained in actual flight. First of all, we were impressed by the fact that there were no errors at all among the pilots. The number of errors was significant, 41.3%, in operators, 9.7% being referable to determination of heading and glide path in the presence of bank, 18.4% to determination of direction of bank, 2.6% to direction of deviations from heading and 10.6% to glide path. As we see, there was prevalence among operators of errors in determining the direction of deviations, particularly according to bank. This occurred in actual flight, when not only the ground, but the wing of the aircraft were visible through the cockpit window. The latency period of the verbal response was noticeably shorter for pilots (Figure 2).

The differences in output characteristics of pilot and operator actions cannot be attributed to chance. They are determined by differences in internal content of actions directed toward evaluation of spatial position. The typical nature

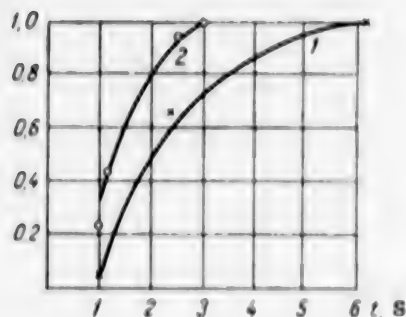


Figure 2.

Functions of distribution of latency time of restoration of spatial orientation

1) operators 2) pilots

spatial position in pilots and operators during flight. In operators, the image corresponds to the view of the world around them as seen from the aircraft window, i.e., the conception (image) coincides with visual perception. In pilots, the spatial relationships are transformed and viewed on the basis of a geocentric system of coordinates. The specifics of content of the pilot's conceptual model in flight is what determined their error-free evaluation of spatial position.

Thus, as a result of this study, we obtained the following evidence: 1) the perception image that controls pilot actions in flight differed from that of the operator; 2) the pilot not only conceives of, but perceives displacement of the aircraft (himself) in relation to the stationary ground-based reference points and the ground.

It is known that, in the course of ontogenetic and phylogenetic development of man, by virtue of coordinated activity of analyzer systems, an image reflecting spatial relations is formed in a geocentric system. However, the unusual factors to which man is exposed in flight destroy the customary orientation system that was formed on the ground. In a pilot (unlike an operator), there is adaptation to altered sensory fabric of the image in the course of flying experience. As a result, his perceptions in flight are adequate to a man's conception that he moves in relation to the stationary ground.

#### Laboratory Study

As can be seen in the Table, addition to the pattern of an IM on the basis of the landing symbol of the artificial horizon line (variant B) reduced the number of errors to almost 1/2. Further increase in graphic elements in the pattern--saturation with perceptive signs of the geocentric system by adding, in addition to the horizon line, signs of depth (variant C), outline of runway (variant D) and then the silhouette of the aircraft (variant E) also--reduced significantly the number of errors.

We are dealing with errors related to egocentric orientation, use of which is provoked by a given IM. The typical nature of erroneous decisions and their genesis were described previously in detail [2].



Efficiency and reliability of pilots' spatial orientation as a function of presence in IM of perceptual signs of external space

Parameter analyzed	Variant of information model				
	A	B	C	D	E
Relative number of errors, %	42,3	23,3	20,5	17,6	2,5
Latency period of assessing spatial position, s	3,4 (2,0—4,1)	2,5 (1,5—3,0)	2,2 (1,1—3,0)	2,3 (1,1—3,0)	2,1 (1,0—3,0)

The latency period diminished reliably ( $P < 0.05$ ) with use of information models in variants B, C, D and E, as compared to A. Subjectively, all pilots reported that variants D and E facilitated appreciably evaluation of the aircraft's spatial position, as compared to variants A and B.

Consequently, addition to the IM of graphic elements of external space made it possible to optimize the process of using the geocentric system of coordinates.

What then are the mechanisms of effect of this factor on choice of center of subjective coordinates?

First of all, it must be noted that the process of formation of spatial conceptions of aircraft position on the basis of IM occurs indirectly, i.e., the pilot is not dealing with real objects in the space around him (as in visual flight), but with an image of these objects and their spatial characteristics on the flat screen of the instrument.

Studies established that the distinctive feature of the images is their ambiguity, which occurs due to the fact that in projecting real objects on a plane there is loss of part of the information about them. In this case, one refers more often to dual perception, in the sense that there is perception of visible spatial relations between elements in a flat image, on the one hand, and a more complex process of primary data processing and, on their basis, construction of an idea about the real object from the image, on the other hand [6].

In our study, the landing symbol shown in the IM (variant A) is a projection of extended reference points designating the heading for landing and plane (glide path) of descent. Formation of an idea about the position of the aircraft from the image of the projection of these reference points in simulated situations was associated with errors due to difficulty of mental orientation of the image of the landing symbol in relation to the geocentric system of coordinates, in spite of the fact that the configuration of the symbol indicated the basic directions of this system (horizontal, vertical and sagittal components). Addition to the IM of perceptual signs of space facilitated mental correction of the landing symbol in relation to the geocentric system of coordinates. This was manifested by reduction of number of errors and time spent by pilots on evaluating the spatial position.

Optimization of conditions for formation of spatial conceptions in the case of adding to the IM the symbol for the aircraft is related to manifestation of the phenomenon of figure and background. In essence this phenomenon consists of the fact that the object that is seen as a figure against the background created by another object is perceived as moving [8]. In our study (without the aircraft symbol on the IM), the image of the landing symbol becomes a figure on the screen. Addition of the aircraft silhouette to the IM caused change in correlations between figure and background, since in this case the image of the landing symbol serves as the background and the silhouette serves as the figure. This circumstance is what caused formation of spatial conceptions in the pilot in a geocentric system of coordinates. It must be noted that the "from the ground to aircraft" type of display (stationary horizon line with regard to bank) is more reliable for orientation than "from aircraft to the ground" (horizon line is mobile for bank), since the aircraft symbol actually moves in relation to the image of external reference points. As a result, there are no conditions to perceive mobility of the image of these reference points in relation to the instrument panel and the pilot. Researchers have mentioned expressly this circumstance to explain the motor reversal errors encountered among pilots when using a gyro-horizon with "from aircraft to the ground" type of display [3, 9, 10]. Consequently, when discussing optimization of formation of spatial conceptions in an expedient geocentric system of coordinates, conditions of unambiguous perception of aircraft movement with use of "from ground to aircraft" type of display are more helpful than use of IM with "aircraft to ground" type of displays.

Thus, the results of these experiments revealed that use by man in flight of a purposeful (geocentric) method of orientation is determined, in the first place, by the specific content of the conceptual model formed in the course of flight experience and, in the second place, fullness of expression in the IM of signs (elements) of a geocentric system of coordinates.

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EFFECT OF THREAT STRESS ON PSYCHOMOTOR STABILITY OF PILOTS DIFFERING IN ANXIETY LEVEL

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 16 Mar 83) pp 24-26

[Article by J. Terelak and J. Maciejczyk (Polish People's Republic)]

[English abstract from source] The apparent anxiety as a personality factor cannot serve as a differentiating factor in the performance of psychomotor tasks (oculomotor coordination). It is shown that when pilots manifest a higher level of anxiety, performing specific tasks, their physiological expenditures increase.

[Text] The problem of the effect of stress on pilot performance is important in aviation psychology. For this reason, many theoretical and experimental investigations are conducted within the limits of this discipline [11].

We selected out of the abundant range of problems, the reaction to stress elicited by a threat [6] as the object of experimental investigation. Electric current was used as a stressor, since its physical distinctions make it possible to effect monitoring and do not cause any physiological damage. Electricity is an independent variable. A dependent variable is referable to the area of visual and motor coordination. The indirect variable pertained to a personality trait such as anxiety.

Thus, the tested sensitivity to stress can be defined as an emotional reaction (reactivity) to a threat (electric current), manifested on both the physiological (pulse rate) and behavioral (psychomotor stability) levels.

There have been numerous experimental studies, in which the authors tried to demonstrate the relationship between electric stimulation, personality traits and psychomotor stability [1, 2, 4, 5]. These studies revealed that there are no similar results in solving different problems in a stress situation. Some actions are characterized by elevation of recorded parameters and others, by decline.

In the opinion of many authors, anxiety, or alarm [12], is one of the factors that modifies the effect of stress. On this basis, we adopted the following



hypothesis in our study: pilots with high level of anxiety must show a greater decline of psychomotor indicator under the effect of stress induced by a threat (electric shock as a punishment) than pilots with low anxiety level. It is believed that the differences should affect, first of all, energy expenditures of the body, which is manifested in particular by a change in pulse rate.

## Methods

We also used an aircraft simulator to determine changes in structure of instrumental function. The subjects' task was to visually track a light spot on the simulator's screen, which changed its position in accordance with a program, as well as to perform corrective actions using levers and pedals in order to hold the spot in the crossbeam, i.e., center of the screen. The number of corrective hand (lever) and leg (pedal) movements was a criterion of achievement. We also measured pulse rate (average reading time 1 min).

Stimulation with electric current (500  $\mu$ A, 0.01 s) was used to determine the effect of a stress situation of the threat type on the process of psychomotor actions; it was delivered by means of two electrodes situated on the forehead of the subject. Distance between electrodes was 5 cm.

According to the instructions, electric stimulation was to serve as a punishment when the light spot moved 1 cm away from the center of the screen.

In actuality, the current was used only three times in each stress test. The subjects became familiar with the effect of the stimulus before the stress tests. We tested two groups of supersonic aircraft pilots 25-40 years of age. The individuals in the 1st group (20 people) were characterized by a high anxiety level, as determined (before the test) by means of a questionnaire. The subjects in the 2d group (control, 20 people) had a low anxiety level.

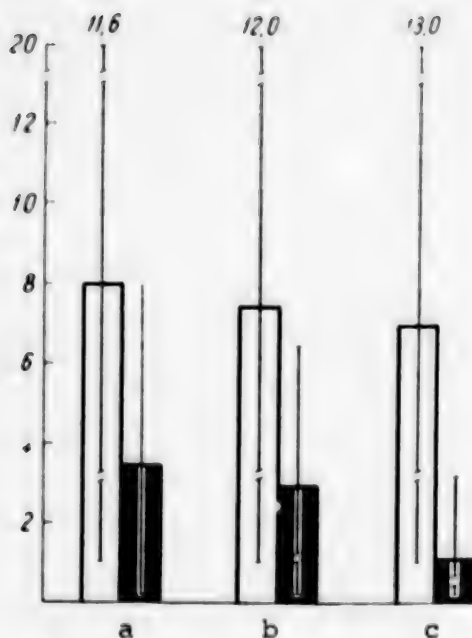
The tests were performed under identical laboratory conditions in the period from 0900 to 1200 hours. Each subject performed seven tests. The first was an adjustment test, the next two were stress tests (subjects were punished with electric current) and the remaining four were performed after the stress tests (electricity was not used).

## Results and Discussion

The results were submitted to statistical processing using the Student test. The performance results are illustrated in the Figure.

This figure shows that there was no statistically reliable difference in achievement parameters between the groups. At the same time, there was definitely a wider (about 50%) scatter of results in the 1st group. This means that there is a tendency toward increased sensitivity to stress among pilots with high anxiety level.

The results coincide with those of many authors [3]. Sarason [13] observes that the lack of appreciable difference in performance results between individuals with high and low anxiety levels could be due to the difficulty of



Differences in simulator task performance between first (white bars) and second (black bars) groups; y-axis, number of exits from center

a, b, c) base, stress and post-stress tests

the problems. When solving more difficult problems one usually observes worsening of performance under the influence of stress and when solving easy ones, an improvement is observed. Sarason's comments confirm the results of our tests, where there was improved performance in subjects of both groups (particularly the first).

Training to suppress anxiety, control of one's emotions in a stress situation, which is acquired in the course of pilots' professional activities, is another cause of lack of difference in achievement level.

Pulse rate before the test (at rest), prior to each test (base frequency) and during each test (for 45-s periods) was the physiological parameter of anxiety level. The Table lists the results of analysis of level of activation, an indicator of which was pulse rate.

This table shows that the parameters of individuals in both groups differed reliably in pulse rate at rest (91.3/min in the 1st group and 82.0 in the 2d) without differences in base studies, during the stress tests (with electric current) and

after them. The pulse rate held at a relatively high level in the first group, as compared to the control. We were impressed by the fact that the pulse rate rose at the start of the test in both groups (particularly the control). Analogous findings were made by Lazarus in tests with electric current [9]. Adaptation occurs during the tests. This is confirmed by the results of other authors [7, 10].

#### Results of analysis of activation level (pulse rate)

Test	Arithmetic mean	Dis- persion	Standard deviation	<i>t</i>	<i>p</i>
First group					
Base	91,3	210,71	14,516	0,4862	unreliable
	93,6	216,05	14,699		
Stress	91,3	210,71	14,516	0,2240	"
	92,4	248,38	14,699		
Poststress	91,3	210,71	14,516	0,1173	"
	90,8	136,07	11,665		
Second group					
Base	82,0	170,30	13,050	3,5100	<0,01
	91,6	142,14	11,922		
Stress	82,0	170,30	13,050	1,7487	unreliable
	89,1	143,90	11,996		
Poststress	82,0	170,30	13,050	1,7456	"
	89,0	136,46	11,682		

In summarizing our arguments, it should be noted that the lack of statistically reliable differences in performance of psychomotor tasks by pilots of the tested groups is attributable to the relatively simple structure of the task, on the one hand, and characteristics of the population, on the other. As we know, pilots are characterized by significant endurance of threat stress, as manifested by the ability to suppress negative emotional states. Moreover, stress is not only a source of fear, which disorganizes action, but the source of stimulation that could increase productivity of work.

There are individuals characterized by stability in stress situations [8, 14]. We can include among them pilots who, as a result of psychological screening and professional training, retain better stability of action in stress situations.

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USE OF FLIGHT SIMULATORS FOR DEMONSTRATION OF FUNCTIONAL CAPACITIES OF FLIGHT PERSONNEL

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 27 Jan 84) pp 26-29

[Article by V. A. Bodrov, A. A. Kupriyanov, A. G. Fedoruk and V. V. Kharin]

[English abstract from source] Psychophysiological parameters of 89 pilots were examined when they performed flight tasks under normal and complicated conditions. The experiments helped to reveal a group of test subjects (11.2%) who had low capabilities and made serious errors. The results obtained suggest that psychophysiological examinations during stimulated professional activity can be recommended as a method to be used for measuring adaptive and compensatory capabilities of pilots undergoing medical expertise.

[Text] The physical loads of flight personnel did not diminish with use of modern aviation complexes, whereas the mental loads increased to such an extent that the limit of human capacity is reached under some flight conditions. This causes increase in number of functional diseases of the nervous and cardiovascular systems [3]. Health deviations have an adverse effect on inflight pilot work capacity, particularly in difficult and emergency situations [5]. The opinion is held that it is possible to demonstrate the functional capacities of pilots and to predict them under conditions as close as possible to actual working conditions. For this reason, efforts have been repeatedly made to conduct psychophysiological examinations of flight personnel during performance of flight assignments in a flight simulator (FS) [2, 4, 6, 8]. In some cases, use of the proposed method made it possible to solve some problems of differential diagnosis. However, no conclusions were drawn as to the functional capacities of pilots. In addition, the lack of a standard approach or criterion to assess the psychophysiological aspect of flight assignments and methods of evaluating the quality of their performance did not permit, until now, broad use of pilot examination on FS in the interests of expert medical certification of flight personnel. All this makes it necessary to develop and use the work-load test which simulates the basic elements of an actual flight and the most probable complications in addition to the methods used in the practice of expert medical certification of flight personnel. Such a method should demonstrate the degree of a pilot's adaptation to his professional work. The present investigation of functional capacities of



some categories of flight personnel during performance of flight assignments on an FS was conducted on this basis.

#### Methods

Standard FS were used for the investigation.

In order to obtain comparable data, the flight assignments provided for the pilots to work only with cockpit equipment that they knew. Before the test, the pilot was informed about the nature of the flight assignment and he was allowed to make some preliminary practice flights. After reporting that he was ready, the study was performed and it included the following: performance of 4 landing approaches in 2 180° turns at an altitude of 600 m and speed of 600 km/h; flight into the area for making turns with a  $\pm 60^\circ$  bank at an altitude of 2000 m and speed of 700 km/h; gaining altitude from 600 to 2000 m and descending from 2000 to 600 m at a speed of  $\pm 10$  m/s; coping with a complicated situation produced by covering the flight instruments with a special blind, with subsequent simulation of various spatial positions of the aircraft (at different altitudes, bank and pitching angles, vertical and instrument speeds).

In addition, when making the second landing approach, turning in the zone, gaining altitude and descending, complications were presented to the pilot: instrument malfunction, cross wind during landing, request to perform arithmetic problems verbally, associated verbal test with inclusion of words with general, professional and emotional meaning related to prior accidents and diseases.

These complications were added in order to demonstrate changes in structure and psychophysiological distinctions of pilot performance, as well as to determine his resistance to interferences. They can be arbitrarily divided into specific and nonspecific ones. Specific complications refer to factors that correspond to the pilot's working conditions (malfunctions of various systems and units, cross wind, difficult spatial positions, etc.). Nonspecific complications refer to various mental loads in the form of arithmetic problems. Nervous and mental stress were produced by the suddenness of appearance of a complication and need to make an immediate decision.

Parameters of physiological functions and quality of performance were recorded when the crossbeam passed on the runway, as well as on the descent glide path. Heart rate (HR), phonogram, respiration rate (RR) and minute volume (MV), galvanic skin response (GSR) and EMG of right arm flexors were recorded using an electroencephalograph and the Physiologist-3M instrument.

Nervous-emotional stress was assessed on the basis of the increment in recorded physiological parameters as compared to the resting state and background flights (without complicating work) [1].

Performance quality was recorded by means of taking motion pictures of flight instrument readings on the instructor's console using an RFK-5 camera at the rate of 1 frame per second. To evaluate the pilot's actions to correct a difficult situation, movements of controls and readings of flight parameters were recorded on a K-20 oscillograph. In addition, the flight route was

entered on the plotting board. Quality of performance was assessed by the existing standards [7]. Efficiency of pilot actions to correct the complicated situation was evaluated by the time from the moment the blind was removed from the pilot's instrument panel to the first correct movement of the control to counteract the deviations, as well as the end result, i.e., bringing the "aircraft" into horizontal flight or "ejecting" at a safe altitude.

The method we developed was used to test 89 pilots undergoing routine certification in a hospital. The subjects were divided into 3 groups according to diagnosis: the 1st group consisted of 31 people without diagnosis, the 2d--21 with functional diseases of the cardiovascular system, 3d--37 people with functional diseases of the nervous system. All of the tested pilots were deemed fit for flight work according to the conclusion of the expert medical commission for certification of flight personnel.

### Results and Discussion

The studies established that the 2d and 3d groups of pilots are under greater nervous and emotional stress when performing tasks on the simulator.

This is indicative of the possibility of detecting functional deviations in the main physiological systems during simulation of professional activities. Particularly distinct differences between parameters of the examined pilots are demonstrable at the most difficult stages of flight, for example, in heart rate when making a landing approach (Figure 1).

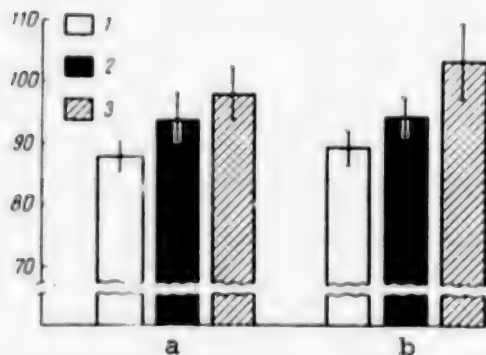


Figure 1.

HR parameters in pilots during stages of landing approach

a) OHS      b) IHS

Here and in Figure 2:

1-3) 1st-3d groups of pilots, respectively

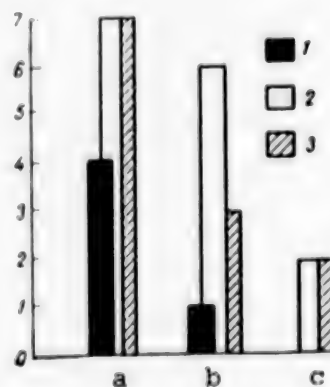


Figure 2.

Total number of flight parameters that were not kept at set levels during four landing approaches by pilots

a) 12 km distance  
b) OHS  
c) IHS

There are also substantial differences in quality of professional performance by pilots of the 1st group and those in the 2d and 3d groups. The performance of all flights made by the 1st group of pilots was rated as an average of 3.8, and for those in the 2d and 3d groups, 3.4-3.6. In addition, the latter

went beyond the set standards for flight parameters twice as often. We should call attention (Figure 2) to the fact that, by the time they made the decision to land (passing the inner homing station--IHS), the 1st group of pilots showed virtually no deviations of flight parameters from the established standards, whereas those in the 2d and 3d groups presented deviations of an average of 2 parameters.

Mean time (M±m) of performance of main actions on FS to eliminate complicated situation (altitude at which blind was opened 2000-3000 m)

Group of subjects	Latency period of 1st movement	Bank correction time	Return to horizontal flight time	Total time of elimination of complicated situation
1	2,3±0,4	6,7±0,7	7,6±1,0	16,6±1,4
2	1,7±0,2	7,6±0,7	15,0±2,3	24,3±2,2
3	2,2±0,3	6,7±0,5	13,1±1,8	22,0±2,1

The results of pilot actions on the FS to correct difficult situations (see Table) indicate that total time required for flight personnel with deviations of health status was 32-46% longer than for healthy pilots. This shows that there was decline of their functional capacities in difficult situations, which could lead to piloting errors in actual flight.

In addition, it was found that in all three groups of pilots there were some whose parameters of physiological functions were considerably higher than the means for their groups. For example, in the 1st group there were pilots whose HR reached 120/min (mean group parameter 89/min) at the stage of passing over outer homing station (OHS), it was 123/min in some pilots of the 2d group (mean group parameter 94/min) and 138/min in some of the 3d group (mean 98/min). These data indicate that there are individuals with heightened reactivity among the pilots in all 3 groups (they constituted 11.2% of all those tested). The parameters of their physiological functions differed appreciably at all stages of FS flight from the means for the corresponding groups. Mean HR constituted  $118 \pm 2.8$ /min when passing over the OHS for the subjects with high reactivity, versus 93.6/min for all tested pilots. For the sake of comparison, let us mention that, according to data in the literature, the top range of HR for this phase of flight is 95/min with the FS and  $112 \pm 12.2$ /min in an actual flight.

It is known that excessive pilot stress in flight leads to decline of his functional capacities. Such pilots are more susceptible to depletion of functional reserves and failures in their professional performance. This is confirmed by the results of examining pilots in the high reactivity group. For example, their performance of flight assignment on the simulator was rated as "poor" and they made more gross errors.

Thus, the results of examinations with flight simulation on FS permit evaluation of functional capacities of flight personnel and help make validated expert decisions.

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EFFECT OF LEVEL OF PHYSICAL ACTIVITY ON LIPID METABOLISM OF FLIGHT PERSONNEL

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 15 Nov 83) pp 29-31

[Article by V. I. Libkind and V. D. Vlasov]

[English abstract from source] Lipid metabolism of healthy pilots was investigated and verified by the PWC<sub>170</sub> test. Pilots with a lower physical activity showed high levels of cholesterol, triglycerides, total lipids, LDL and low levels of HDL as averaged per group. The PWC<sub>170</sub> value and the concentration of lipid fractions in blood were found to be highly correlated, the correlation being nonlinear. The nonlinear regression data suggest that greater physical activity leads to a decrease of the lipid concentration and an increase of the content of high density lipid-protein complexes.

[Text] The question of link between lipid metabolism and level of physical activity is important, not only from the standpoint of investigating the pathogenetic aspects of onset of cardiovascular disease. The urgency of the problem is due to the increasing significance of the cardiological factor in aerospace medicine, as well as the need to institute preventive measures for flight personnel in order to improve the reliability of piloting flight vehicles [5].

We submit here the results of testing some parameters of lipid metabolism in flight personnel differing in level of physical activity, as verified by an objective method.

#### Methods

We examined 105 healthy pilots 22-40 years of age. Their physical activity was evaluated by determining physical work capacity using the PWC<sub>170</sub> test on a bicycle ergometer. The variation statistical method of examining the results of performing this test was used for gradation of its level in flight personnel, and it permitted separation of subjects into 3 groups. Individuals whose PWC<sub>170</sub> was in the range of  $\bar{X} \pm 1$  were arbitrarily considered the norm [2] and referred to the pilot group with average physical work capacity. Those with parameters in excess of  $\bar{X} + 1$  and below  $\bar{X} - 1$  constituted the groups with good and low work capacity, respectively. The three professionally homogeneous groups formed

as a result of distinctive separation were of approximately the same average age and identical in life style, job, qualifications and work tenure.

The following lipid metabolism parameters were determined: total lipids according to Zollner, total cholesterol according to Ilk, triglycerides according to Carlson-Ignatovskaya, lipoprotein fractions--chylomicrons (CM), very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), high-density lipoproteins (HDL) by disk electrophoresis in polyacrylamide gel as modified by Ye. A. Magracheva.\*

The data were submitted to statistical processing by the method of regression-correlation analysis using a YeS-1033 computer [4].

### Results and Discussion

In spite of the fact that the mean group age was the same, the subjects' diet was similar and the nature and conditions of their professional activities were identical, mean blood levels of lipid metabolism components differed with statistical reliability. Highest mean group values for total cholesterol, triglycerides and total lipids were found in pilots with low physical work capacity. Total cholesterol and triglycerides constituted 4.30 and 0.88 mmol/l, respectively, in subjects with good physical activity. In those with lower physical activity these parameters constituted 6.01 and 1.89 mmol/l ( $P < 0.001$ ). There were statistically reliable differences in mean group lipoprotein levels. Higher LDL and the lowest HDL values were observed in pilots with low physical activity.

Thus, we demonstrated definite changes in lipid metabolism in subjects with low physical activity (see Table), which increased the danger of development of diseases of atherosclerotic genesis [1, 3].

Results of complete correlation analysis of parameters of lipid metabolism as a function of  $PWC_{170}$

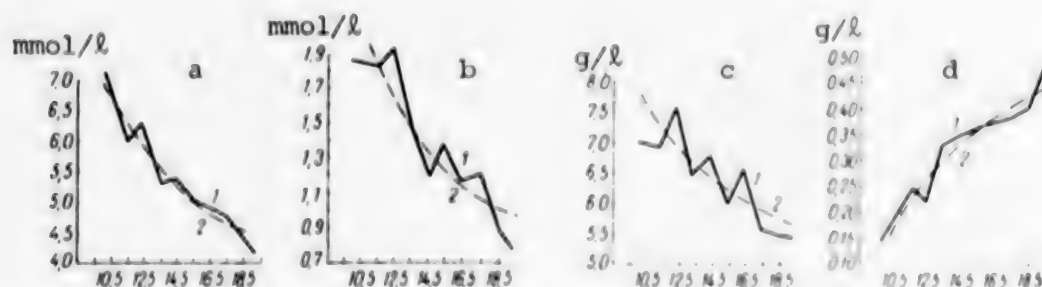
Statistical parameter	Total cholesterol	Tri-glycer.	Total lipids	LDL	HDL
Coefficient of correlation	-0.731	-0.631	-0.433	-0.554	0.612
Coefficient of determination, %	53.4	39.8	18.7	30.6	37.4
Correlation ratio	$\frac{0.896}{0.844}$	$\frac{0.788}{0.713}$	$\frac{0.853}{0.722}$	$\frac{0.974}{0.870}$	$\frac{0.934}{0.866}$
Coefficient of curvilinear correlation	0.870	0.750	0.787	0.922	0.900
Indicator of linearity of relationship	0.222	0.164	0.432	0.544	0.436

Note:  $P < 0.001$  for all parameters.

The results of correlation analysis indicate that there is a statistically reliable link between level of pilot physical activity in terms of  $PWC_{170}$

\*I. M. Aleykin performed the biochemical tests.

parameters and concentration in blood of lipid and lipid-protein fractions (see Table). As in most biological phenomena, the correlation was not so much rectilinear as it was complexly nonlinear. Among the components of lipid metabolism, there was the highest linear correlation demonstrated for total cholesterol. In other instances, when the linear correlation between  $PWC_{170}$  parameters and blood lipid fraction content was insufficiently distinct, the values for correlation ratio and coefficient of curvilinear correlation confirmed the existence of a close link, though more complex in form (see Figure).



Changes in blood lipid content as a function of level of physical work capacity ( $P < 0.01$  according to Fisher's criterion); x-axis,  $PWC_{170}$  (in kg-m/min·kg)

- a) cholesterol
- b) triglycerides
- c) total lipids

- d) HDL
- 1) empirical line
- 2) line of regression

Use of the nonlinear regression method made it possible to demonstrate the nature and dynamics of the link between values for  $PWC_{170}$  and parameters of lipid metabolism. In all instances there was a hyperbolic function. Approximated lines and equations of regression revealed that an increase in  $PWC_{170}$  is associated with smooth, mathematically expected decline in blood concentration of total cholesterol, triglycerides, total lipids and a shift of the lipoprotein spectrum in the direction of increase in high-density lipid-protein complexes.

Thus, the correlations between physical activity and blood lipid content of the subjects are characterized by a number of distinctions. The main ones are the difference in lipid metabolism between subjects differing in level of physical activity, high correlation between lipid fraction content of blood and level of physical work capacity. The findings warrant the belief that an increase in physical activity by flight personnel should result in diminishing the role of the cardiological factor in flight safety from the standpoint of aviation medicine.

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OXYGENATION AND REGIONAL CIRCULATION IN GINGIVAL MUCOSAL TISSUES UNDER EFFECT OF HEAD-TO-PELVIS (+Gz) ACCELERATIONS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 18 Jul 83) pp 31-35

[Article by S. I. Vol'vach, Ye. A. Kovalenko, L. I. Voronin, N. V. Ulyatovskiy, V. K. Gabyshev, V. I. Nikiforov and V. V. Arkhipov]

[English abstract from source] The information content of a new rheopolarographic procedure used to determine the oxygen balance and regional circulation of the gingival mucosa was measured, bearing in mind the applicability of the procedure as an objective index of human tolerance to +Gz acceleration. It was found that the parameters of the oxygen balance and regional circulation of the gingival mucosa were well correlated with blood pressure in the floor of the auricle. In contrast to the traditional methods for assessing tolerance to acceleration, the new procedure provides information about the health condition of the centrifuged subjects on a continuous basis. Variations in the oxygen balance and regional circulation of the gingival mucosa helped to identify compensatory reactions of the cardiovascular system in response to +Gz acceleration.

[Text] In order to assess tolerance to head-pelvis (+Gz) accelerations, it is important to know the distinctions of oxygen supply to brain tissue, which is the overall indicator of degree of anemization and hypoxia of tissues [2]. Use of the polarographic noninvasive method of measuring  $pO_2$  in tissues located in the zone of blood supply of the carotid artery may be of special interest. Unlike oxyhemograms, the polarographic method permits measurement of  $pO_2$  at the last stage of oxygen transport, i.e., directly in tissues. Our objective here was to test and examine the informativeness of a new contact method of testing oxygenation and regional circulation of the gingival mucosa with exposure to head-pelvis accelerations.

#### Methods

This study was conducted on 5 healthy males 25-30 years of age. On a centrifuge with a 7-m arm, 3 series of tests were conducted with exposure to accelerations with a gradient of 0.1 G/s at +2.5 Gz for 5 min, +3.0 Gz for 5 min, +3.5 Gz for 30 s, +4.0 Gz for 30 s and +4.5 Gz for 30 s, with and without using

protective measures--pharmacological and anti-G suit. There was a  $10^\circ$  angle between the acceleration vector and longitudinal axis of the subject. A total of 22 rotations were used.

Maximum tolerance was determined on the basis of loss of peripheral vision (increase in latency period of motor reaction to a photic stimulus --  $\geq 2$  s), appearance of gray veil and spontaneous muscle relaxation, arterial pressure (BP) drop in vessels of the concha ( $< 40$  mm Hg).

We recorded continuously the electrocardiogram (ECG) in the three leads of Nehb, heart rate (HR), BP (minimum, maximum, lateral systolic and dynamic mean) in the brachial artery by the tachoscillographic method, systolic BP in vessels of the concha by the method of P. M. Suvorov (1969), respiration rate (RR), electromyogram (EMG) of femoral and anterior abdominal wall muscles, motor reaction time to photic signals, parameters of oxygenation and regional circulation of the gingival mucosa using a combined rheopolarographic method developed at the Central Scientific Research Institute of Stomatology, USSR Ministry of Health [4], before and during the test, and in the 5th min of the recovery period (RP).

In this study, we used a refined rheopolarographic sensor consisting of two sections applied bilaterally to the tested region of the maxillary gingival mucosa. Each section of the sensor contained a polarographic contact electrode with two active platinum cathodes and a pair of rheographic electrodes--active and for measurement. Thus, the sensor permitted simultaneous registration of  $pO_2$  at 4 points of the gingival mucosa and tetrapolar rheogram [4]. The sensor was attached to the mucosal surface by means of an adhesive coating. We used a silver chloride electrode placed on the upper arm as the silent electrode. The polarographic sensor was connected to an oxymeter (CSSR), polarization current in the tested tissues was recorded on a KSP-4 automatic recorder which was outside the centrifuge (CF). The rheographic sensors were connected to a Rheograph-2, and rheograms were recorded on a Mingograph-34. Polarographic sensors were calibrated in saline before and after the tests. Intensity of blood flow in the tested tissues was assessed by the dynamics of the rheographic index (RI), while oxygenation was evaluated by the dynamics of partial oxygen tension ( $pO_2$ ).

The results of these tests were submitted to statistical processing with use of the nonparametric criteria of Wilcoxon-Mann-Whitney and Spearman's coefficient of rank correlation [1].

## Results and Discussion

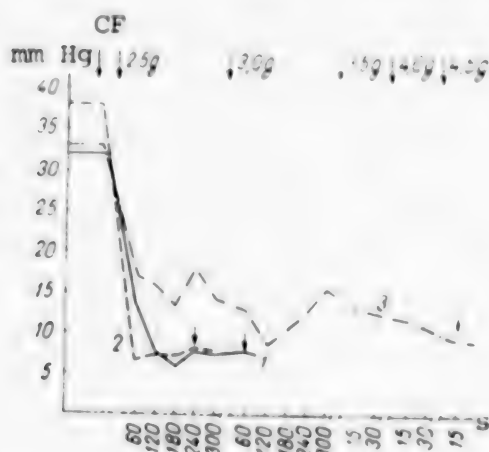
During the first minutes of exposure to  $+2.5$  Gz accelerations, there was decline of gingival mucosal  $pO_2$  and RI to 65 and 53% of base values, respectively. BP in vessels of the concha dropped to 50% of base level and constituted a mean of  $55.0 \pm 6.5$  mm Hg. In the 5th min of exposure to  $+2.5$  Gz,  $pO_2$  and RI of gingival mucosa and BP of vessels of concha decreased to 41.3, 56.0 and 38.4%, respectively, of base values. At this time, BP in vessels of the concha constituted a mean of  $43.3 \pm 3.3$  mm Hg. For the next 3 min of exposure to  $+3$  Gz, BP in conchal vessels was 40 mm Hg. This was associated with further decline of  $pO_2$  and RI of gingival mucosa (Figures 1 and 2).



**Figure 1.**  
Dynamics of  $pO_2$  in gingival mucosa of subjects exposed to head-pelvis accelerations

X-axis, time (s); y-axis,  $pO_2$  (mm Hg).  
Here and in Figures 2 and 3:  
1-3) test series I-III, respectively

Here and in Figure 3, arrowheads near curves indicate descent (rotation stopped)

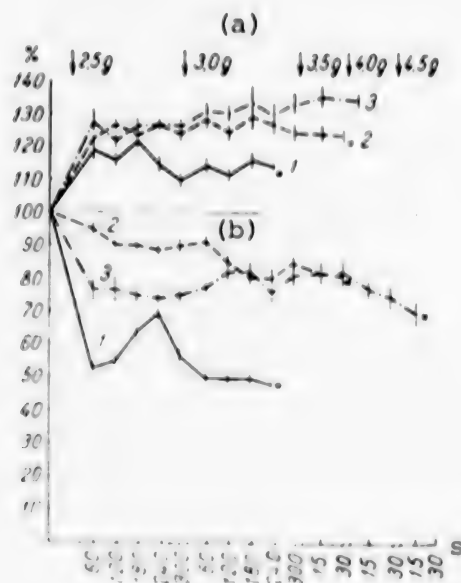


**Figure 3.**  
Dynamics of  $pO_2$  in gingival mucosa of subject Zh. during exposure to head-pelvis accelerations.

X-axis, time (s); y-axis,  $pO_2$  (mm Hg)

the gingival mucosa, as well as BP of conchal vessels, dropped to 81.5, 95.5 and 58.8%, respectively, of base values. Absolute BP in vessels of the concha constituted  $66.6 \pm 13.3$  mm Hg. One must always take into consideration these important findings.

In the 5th min of exposure to +2.5 Gz,  $pO_2$  constituted 61.5% of the base value, RI 89.0% and BP of conchal vessels 50.1% ( $56.7 \pm 8.8$  mm Hg). The observed



**Figure 2.**  
Dynamics of BP (a) and RI (b) in gingival mucosa of subjects exposed to head-pelvis accelerations

X-axis, time (s); y-axis, deviation (%). Asterisks show when rotation was stopped.

At the time the command was issued to stop rotation, due mainly to subjective indications,  $pO_2$  constituted a mean of  $12.2 \pm 3.2$  mm Hg, which constituted 37.9% of base value. Blood flow in the gingival mucosa constituted  $48.8 \pm 5.8\%$  of base level, while BP in conchal vessels was 40 mm Hg.

It is interesting to note that with higher tolerance to accelerations in series II studies, there was considerably less decline of  $pO_2$  and RI of gingival mucosa and BP in conchal vessels. In the 1st min of exposure to +2.5 Gz accelerations,  $pO_2$  and RI of

differences between parameters in series I and II were statistically reliable ( $P < 0.05$ ).

To rule out the influence of the psychological factor due to intake of a pharmacological agent known to enhance tolerance to accelerations, one rotation in this series was performed after giving the subjects placebo. In this case, all of the tested parameters showed virtually no difference from analogous parameters in the same subject obtained in the first series (Figure 3).

In series III, tolerance to accelerations was highest. At the start of rotation,  $pO_2$  and RI of the gingival mucosa declined reliably more than in series II and reliably less than in series I. Thus, in the 1st min of exposure to +2.5 Gz accelerations,  $pO_2$  and RI constituted 70.0 and 77.1%, in the 3d min 54.5 and 75.1%, respectively. In the 4th min of exposure to +2.5 Gz  $pO_2$  and RI began to increase and already by the 2d-3d min of +3.0 Gz these parameters reached values recorded in series II--56.2 and 83.2%, respectively, of base values. With further increase in accelerations,  $pO_2$  and RI of gingival mucosa held at the same levels as in the first minutes of +3.0 Gz. In the preceding series, accelerations in excess of +3.0 Gz were associated with decline of  $pO_2$  and RI, so that the tests had to be stopped for medical indications. In series III, a decline of the tested gingival mucosal parameters started in the 1st min of exposure to +4.0 Gz and rotation was stopped in the 1st min of exposure to +4.5 Gz accelerations. Unlike parameters of oxygenation and regional blood flow of the gingival mucosa, BP in vessels of the concha was reliably higher in series III from the very start of rotation than in the two preceding ones.

In spite of the observed differences in dynamics of parameters of oxygen balance, regional circulation and BP in conchal vessels, there was an overt and reliable positive correlation between these parameters. The coefficient of rank correlation  $\rho$  constituted 0.85 ( $P < 0.01$ ) between  $pO_2$  and BP in conchal vessels, and 0.58 between RI and ear BP ( $P < 0.025$ ).

As we have already indicated, polarization current in tissues of the gingival mucosa, from which  $pO_2$  was calculated, was recorded continuously during rotation. This procedure enabled us to monitor the dynamics of  $pO_2$  in the gingival mucosa during the entire period of exposure to accelerations. In all of the tests, discontinuation of rotation was preceded by drastic decline of the  $pO_2$  curve, which was associated with decline of RI. It is extremely important that this parameter can be used as a prognostic criterion for evaluation of tolerance to accelerations. In cases where  $pO_2$  dropped drastically to 12-15 mm Hg (40-50% of base value) at the start of rotation and there was no compensatory increase of RI of gingival mucosa, one can assume that there was poor tolerance to accelerations. If, however, the  $pO_2$  drop was not so dramatic and was associated with increase of RI of gingival mucosa tolerance could be considered good. Tolerance to accelerations may be determined even without calculating absolute values according to dynamics of  $pO_2$ . Drastic decline of the  $pO_2$  curve and absence of compensatory rises are indicative of poor tolerance to the tested acceleration level. A smoother decline of  $pO_2$  at the start of rotation, presence of compensatory rises during subsequent exposure to accelerations warrant the belief that there is good tolerance. In the course of exposure to accelerations, it is important to differentiate between a decline of  $pO_2$  related to drastic worsening of wellbeing and random fluctuations of this parameter. Of course, one



must take into consideration a number of other concomitant parameters, first of all RI of the gingival mucosa, as well as BP.

We were impressed by the correlation between parameters of oxygenation and regional circulation, on the one hand, and BP of brachial artery, on the other. For example, in series I, concurrently with decline of  $pO_2$  and RI there was increase in all tested BP parameters. When the BP increment reaches a mean of 19.8%, gradual increase of RI of gingival mucosa begins. One minute after reaching maximum increment of tested BP levels, one observes maximum RI increment in this series (to 69% of base value). With further increase of accelerations there is drastic decline of BP increment (to 9.7%), which is associated with dramatic decline of RI (see Figure 2). The observed reaction, which was characterized by increase in RI, was very brief and was not associated with significant elevation of  $pO_2$ .

In series II, exposure to accelerations led to reliable greater increment ( $P < 0.05$ ) in value and duration (as compared to series I) of the tested BP parameters, which was associated with reliably less ( $P < 0.05$ ) decline of  $pO_2$  and RI of gingival mucosa.

In series III, at the first stage of exposure to accelerations, BP increment was virtually the same as in series II, and, as we have already mentioned,  $pO_2$  and RI of gingival mucosa were reliably higher than in series I but lower than in series II. With further increase in accelerations, there was concurrent rise of BP,  $pO_2$  and RI of gingival mucosa. In the 3d min of exposure to 3.0 Gz BP increment reached 33.6%. This is reliably higher than in the two preceding series of studies. In this case,  $pO_2$  and RI of gingival mucosa reached values recorded with the same level of accelerations in series II (see Figures 2 and 3).

Thus, the obtained data graphically illustrate a correlation between blood supply to tissues of the gingival mucosa and dynamics of BP in the brachial artery. This relationship is particularly distinct with use of preventive agents that enhance efficiency of hemodynamics in man.

Thus, the results of this investigation revealed that the combined rheo-polarographic method adequately reflects delivery of blood to tissues situated in the system of the external carotid artery during exposure to +Gz accelerations. A high positive correlation was demonstrated between parameters of oxygenation and regional circulation of the gingival mucosa, on the one hand, and BP in conchal vessels, on the other, as well as a correlation between dynamics of RI of the gingival mucosa and the tested BP parameters recorded for the brachial artery.

Concurrent recording of parameters of oxygen balance and local circulation in the gingival mucosa and parameters of central hemodynamics permits tracking of the mechanism of development of central and local compensatory reactions to redistribution of blood in the body elicited by accelerations.

The results of these studies warrant the belief that parameters of  $pO_2$  and RI of gingival mucosa, together with other parameters of the body's functional state may be used as prognostic criteria of tolerance to +Gz accelerations.

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INVESTIGATION OF SOME ASPECTS OF HUMAN AMINO ACID METABOLISM DURING 120-DAY ANTIORTHOSTATIC HYPOKINESIA

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 6 Dec 83) pp 35-38

[Article by T. F. Vlasova, Ye. B. Miroshnikova and A. S. Ushakov]

[English abstract from source] The free amino acid pool of blood of man exposed to 120-day head-down tilt (at  $-4^\circ$ ) was examined. Beginning with bed rest day 28 and till the end of the study, the amino acid pool increased. The increase involved most free amino acids which was produced by a decline of anabolic and stimulation of catabolic processes during hypokinesia.

[Text] The results of studies with use of restricted motor activity are indicative of the influence of hypokinesia on amino acid metabolism in man [1-4, 6, 8, 12]. Different variants and duration of immobilization lead to some redistribution of the blood's free amino acid pool. Considering the importance of the results of these studies, in developing preventive agents to normalize the blood amino acid balance under hypokinetic conditions, we conducted several studies to assay free amino acids (FAA) in blood plasma during 120-day antiorthostatic [head-down tilt] hypokinesia (AOH, tilt angle of  $-4^\circ$ ).

#### Methods

FAA content of blood plasma of the subjects ( $n = 6$ ) was determined by ion-exchange chromatography on a Liquimat III automatic analyzer (Labotron, FRG) [5, 11]. The tested blood samples were first deproteinized with crystalline sulfosalicylic acid [10]. Venous blood was drawn on a fasting stomach once in the baseline period, 4 times during AOH and 3 times in the recovery period.

#### Results and Discussion

Tables 1 and 2 list the results of examining blood plasma amino acid composition during 120-day AOH and the 14-day recovery period. As can be seen in these tables, there were changes in FAA levels during AOH and in the recovery period. Only cystine and aspartic acid content remained unchanged throughout the period of the study. In the baseline period, blood plasma FAA content virtually failed to differ from the physiological norm [7], with the exception of serine, the concentration of which exceeded the range of physiological fluctuations. On

the 28th day of AOH, the subjects presented a reliable elevation of lysine level ( $P<0.01$ ) and a tendency toward decline of plasma level of threonine against the background of virtually unchanged concentrations of the other amino acids. There was negligible increase in total amino acid pool, which constituted 26.4 mg% (versus 23.5 mg% in the background period). On the 67th-70th days, total FAA content of blood plasma increased by 1.5 times (40.6 mg%). Such elevation of FAA level was attributable to increase in 11 out of 17 amino acids in blood plasma. By the 94th-96th days, the levels of virtually all amino acids exceeded baseline values (with the exception of cystine, aspartic acid and proline). Accordingly, total FAA content increased to 45 mg%, i.e., by 1.8 times, as compared to the baseline period. The concentrations of most FAA of blood plasma also remained high on the 109th-113th days of the study. Leucine, phenylalanine, cystine, aspartic and glutamic acids were exceptions. Total amino acid content at this time constituted 39 mg%, i.e., it was higher than in the baseline period but lower than on the 94th-96th day of the study.

Table 1. FAA content (mg%) in blood plasma of subjects during 120-day AOH (tilt angle  $-4^\circ$ ),  $M \pm m$

Amino acid	Baseline period	Days of AOH				
		28	67-70	94-96	109-113	120
Isoleucine	$0.76 \pm 0.06$	$0.82 \pm 0.08$	$1.36 \pm 0.10^{***}$	$1.58 \pm 0.16^{***}$	$1.11 \pm 0.09$	
Leucine	$1.1 \pm 0.21$	$1.42 \pm 0.13$	$2.99 \pm 0.1^{***}$	$3.10 \pm 0.22^{***}$	$1.48 \pm 0.06$	
Valine	$2.01 \pm 0.30$	$1.97 \pm 0.16$	$2.78 \pm 0.40$	$3.51 \pm 0.19^{**}$	$3.15 \pm 0.24^{**}$	
Threonine	$1.04 \pm 0.14$	$1.22 \pm 0.13$	$1.69 \pm 0.1$	$2.54 \pm 0.15^{**}$	$2.20 \pm 0.24^{***}$	
Serine	$4.40 \pm 0.54$	$3.70 \pm 0.43$	$5.50 \pm 0.6$	$7.12 \pm 0.39^*$	$7.02 \pm 0.44^*$	
Methionine	$0.31 \pm 0.05$	$0.23 \pm 0.01$	$0.68 \pm 0.1^{***}$	$0.69 \pm 0.04^{**}$	$0.52 \pm 0.03^{**}$	
Tyrosine	$0.90 \pm 0.12$	$0.96 \pm 0.08$	$1.47 \pm 0.08^{***}$	$1.79 \pm 0.06^{***}$	$1.65 \pm 0.12^*$	
Phenylalanine	$1.06 \pm 0.22$	$1.02 \pm 0.15$	$1.76 \pm 0.30^{***}$	$1.63 \pm 0.16^{***}$	$1.26 \pm 0.09$	
Cystine	$0.95 \pm 0.17$	$1.18 \pm 0.19$	$1.13 \pm 0.13$	$1.20 \pm 0.12$	$0.85 \pm 0.10$	
Aspartic <sup>a</sup>	$0.91 \pm 0.21$	$0.80 \pm 0.10$	$1.05 \pm 0.10$	$1.14 \pm 0.13$	$0.70 \pm 0.09$	
Glutamic <sup>b</sup>	$1.13 \pm 0.17$	$1.29 \pm 0.17$	$2.76 \pm 0.13^{***}$	$2.08 \pm 0.27^{***}$	$1.83 \pm 0.27$	
Proline	$1.89 \pm 0.12$	$1.71 \pm 0.15$	$2.68 \pm 0.18^{***}$	$2.28 \pm 0.18$	$2.72 \pm 0.13^{***}$	
Glycine	$1.23 \pm 0.14$	$1.32 \pm 0.10$	$2.05 \pm 0.24^{**}$	$2.31 \pm 0.10^{**}$	$2.19 \pm 0.20^*$	
Alanine	$2.04 \pm 0.26$	$2.29 \pm 0.21$	$3.93 \pm 0.39^*$	$4.17 \pm 0.19^{**}$	$2.81 \pm 0.22^{***}$	
Lysine	$2.12 \pm 0.31$	$3.87 \pm 0.44^*$	$4.52 \pm 0.12^{***}$	$5.01 \pm 0.66^*$	$3.98 \pm 0.25^{***}$	
Histidine	$1.18 \pm 0.13$	$1.22 \pm 0.17$	$2.21 \pm 0.36^{***}$	$2.30 \pm 0.06^{**}$	$2.35 \pm 0.14^{**}$	
Arginine	$1.57 \pm 0.30$	$1.40 \pm 0.20$	$2.06 \pm 0.24$	$2.47 \pm 0.27^{**}$	$3.12 \pm 0.15^{***}$	
Total amino acids	25.3	26.4	40.6	45.0	39.0	

Note: Here and in Table 2, 1 asterisk-- $P<0.01$ , 2-- $P<0.02$ , 3-- $P<0.001$ , 4-- $P<0.05$ , 5-- $P<0.02$ ; superscript (a) indicates inclusion of asparagine and superscript (b), inclusion of glutamine.

Throughout the recovery period, which lasted 14 days, complete restoration of base amino acid equilibrium in blood plasma did not occur. Thus, on the 1st day of the recovery period total amino acid content was 40 mg%, on the 5th day 30.8 mg% and on the 14th day 39.9 mg% of baseline values. Only the concentrations of serine, methionine, glycine and arginine came close to baseline



values. Cystine and aspartic acid levels did not change throughout the study. The levels of the other blood amino acids exceeded the baseline in absolute values.

Table 2. FAA content (mg%) in blood plasma during recovery period after AOH, M±m

Amino acid	Recovery period days		
	1	8	14
Isoleucine	0,98±0,04*	1,11±0,05**	1,40±0,13*****
Leucine	1,69±0,33	1,64±0,24	2,14±0,09*****
Valine	3,09±0,13*	2,53±0,22	3,01±0,08*
Threonine	2,68±0,58	2,11±0,21	2,68±0,20
Serine	6,25±0,10****	4,47±0,35	5,25±0,42
Methionine	0,62±0,14	0,44±0,21	0,52±0,07
Tyrosine	1,53±0,47	0,92±0,11	1,55±0,05***
Phenylalanine	1,56±0,43	1,18±0,19	1,68±0,10****
Cystine	0,84±0,07	1,29±0,16	0,97±0,11
Aspartic acid	0,77±0,23	0,45±0,14	1,09±0,09
Glutamic acid	2,83±0,21***	1,94±0,13	1,99±0,11*****
Proline	1,79±0,52***	1,94±0,13	2,96±0,21*****
Glycine	2,26±0,01*	1,40±0,13	2,32±0,14
Alanine	3,18±0,35****	2,33±0,08	3,06±0,18*
Lysine	4,80±0,31***	4,24±0,01***	4,72±0,33***
Histidine	2,53±0,28*****	2,09±0,31****	2,77±0,12***
Arginine	2,63±0,28****	1,80±0,31	2,34±0,28
Total amino acids	40,0	30,8	39,9

The direct and indirect data accumulated to date indicate that the intensity of synthesis of tissue proteins decreases and their degradation increases under hypokinetic conditions, and the severity of changes depends on duration of hypokinesia [9]. During the 1st week, hypokinesia, which acts as a stress stimulus, elicits increase in selective utilization of FAA, leading to decline of the amino acid pool in blood. At later stages, on the contrary, depression of anabolic processes due to diminished adaptation capacity of the body leads to accumulation of blood FAA. Indeed, in tests with 7-day AOH on humans, a decline was demonstrated in total blood amino acid content, which confirmed the hypothesis that there is increased utilization of the body's reserve--FAA--at the early stages of stress, due to their intensive involvement in numerous reactions of intermediary metabolism in the presence of diminished intensity of biosynthetic processes [2]. At later stages of hypokinesia, as was shown with 49-day AOH and during 45- and 120-day clinostatic hypokinesia, there is gradual increase in pool of blood FAA due to depression of anabolic and prevalence of catabolic processes [1, 3, 4, 8]. The results of this investigation also served as confirmation of the previously expounded hypothesis [9]: 120-day AOH, starting on the 28th day, elicited elevation of levels of some blood plasma amino acids. In addition, the gradual elevation of tyrosine level also confirmed development of catabolic processes. Evidently, catabolic processes attained maximum development by the 98th day of AOH, since this is when tyrosine content of blood plasma was at a maximum. Throughout the investigation, as was the case with 49-day AOH [3], the concentrations of cystine and aspartic acid remained unchanged, which is apparently related to their low utilization

under hypokinetic conditions. A 14-day recovery period was not sufficient for normalization of amino acid equilibrium in blood.

Thus, the results of this study of human blood plasma FAA during 120-day AOH indicate that, starting on the 28th day, there is increase in the amino acid pool of blood, as compared to the baseline period due to increase in concentrations of most FAA. The latter is most probably attributable to decrease in intensity of anabolic and prevalence of catabolic processes under hypokinetic conditions.

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## EFFECT OF 7-DAY IMMERSION HYPOKINESIA ON CHARACTERISTICS OF PRECISION MOVEMENTS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 6 Mar 84) pp 38-42

[Article by L. S. Grigor'yeva and I. B. Kozlovskaya]

[English abstract from source] Experiments were carried out to study the effect of 7-day immersion hypokinesia on precision movements that included reproduction of a certain isometric strain of leg muscles (30% from the maximum voluntary level) and angular displacement of the ankle ( $15^\circ$ ) relative to the  $90^\circ$  position. On the first postimmersion day the precision level deteriorated, i.e., the error and variability of the movements to be reproduced consistently increased. The precision decline was the highest with plantar flexion, when the error of the effort and position reproduction was 24 and 28%, respectively, and the lowest with dorsiflexion, when the error was 17 and 25%, respectively, versus 6-8% in the baseline tests. Precision disorders were seen in the structure of movements which lost their stereotypic pattern and became fragmentary, transforming into slow approximate movements versus the pattern of preimmersion movements that were of a rapid programmed control type. Precision changes during plantar flexion movements were usually excessive, hypermetric and almost twice longer than preimmersion. The origin of the above precision changes seems to be primarily associated with muscle atonia. At the same time data analysis shows that in nearly 50% of cases the values of precision changes in movements of various types (efforts and displacements) and different directions (plantar and dorsiflexion) were correlated. This is suggestive of common central mechanisms underlying their development.

[Text] Studies pursued in prior years revealed that exposure to weightlessness and conditions that simulate it is associated with development of functional disturbances referable to coordination mechanisms. This is manifested by change in structure of locomotion, longer performance of motor tasks, lower precision of reproduction of muscular exertions, increased number of errors and variability of characteristics of motion [2, 3, 6-9]. The nature of

changes in coordination and their quantitative relationship to different aspects of effects of weightlessness have not been disclosed to a significant extent. At the same time, knowledge of the pathogenesis and quantitative manifestations of coordination changes is important in order to select precision characteristics of given modes of activity and to develop adequate prevention of disturbances. Proceeding from the foregoing and to supplement previous studies [4], we investigated here the kinematic characteristics of voluntary precision movements.

## Methods

We used "dry" immersion hypokinesia [5] as a test factor.

The study was conducted on 17 healthy men 26-35 years of age, who were tested before, as well as on the 1st and 3d days after 7-day immersion.

The subjects were given two motor tasks: to reproduce from memory graded isometric contractions of crural muscles (30% of maximum voluntary level) and angular motions (15°) of the ankle joint. The tests were performed using an isokinetic dynamometer, which permits standardization of leg position (when movement is performed mainly by the tested group of muscles), and selection of velocities that are adequate to the purposes of the test. Testing was performed with the subjects in supine position: the foot was attached to the dynamometer pedal in such a way as to have the axis of the ankle joint coincide with the axis of pedal rotation, and angles of 120-130° in the hip and knee joints. In the course of the test, the subjects were taught to perform the tasks using visual feedback. For this purpose, 1 (when working in isometric mode) or 2 (when making angular motions) beams were flashed on an IM-789 monitor, showing the specified level of exertion or displacement (Figure 1). According to task conditions, the subjects had to superpose another beam reflecting the exertion or displacement upon the master beam. When the subjects performed their task correctly 5 times in a row, they performed the next 6-8 from memory.

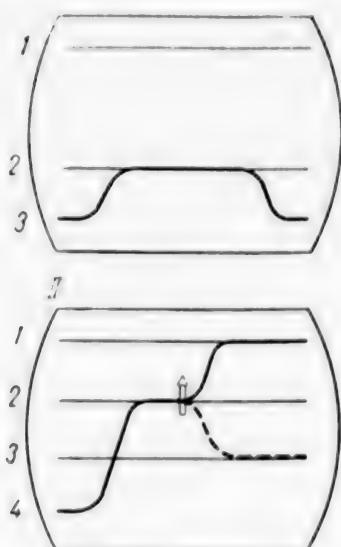


Figure 1.

Diagram of motor tasks

- I) isometric contraction
- II) angular movement

Thin lines--guide beams, boldface, controllable beam

In I:

- 1) maximum voluntary
- 2) 30% of maximum
- 3) trajectory of motion

In II:

- 1, 2, 3) specified positions of ankle joint--75, 90 and 105°, respectively
- 4) trajectory of movement from position of complete relaxation until moving beam reaches mark (arrowhead in center of screen), then to the required position of dorsiflexion (solid line) or plantar flexion (dash line)

force and in the second, angular displacements. When processing the data, we analyzed the magnitude and direction of error, variability of reproduction and



performance time. Reliability of demonstrated differences was determined using Student's criterion.

## Results and Discussion

The results of this study revealed that immersion hypokinesia alters significantly the precision characteristics of movements. As can be seen in Figure 2, the subjects performed both tasks rather precisely in the base state; magnitude of error and variability of reproducing exertions did not exceed a mean of 5-8% and that of positions, 6-10%; the parameters of precision of plantar flexion and dorsiflexion did not differ appreciably.

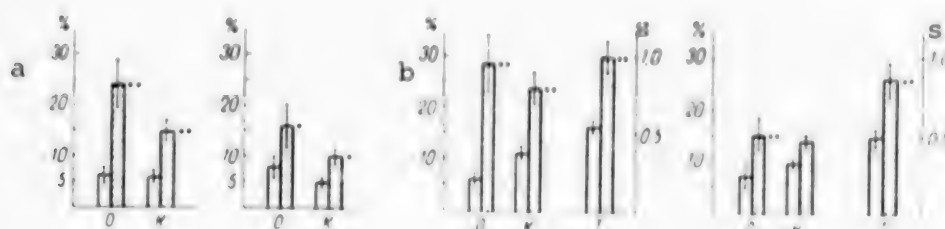


Figure 2. Error (O, %), variability (K, %) and duration (T, s) of reproduction of specified exertion (a) and displacement (b) before and after immersion hypokinesia

On the left, plantar and on the right, dorsal flexion of foot. White bars--before immersion, striped--1st postimmersion day. Vertical segments--standard error; one and two asterisks-- $P < 0.05$  and  $< 0.01$ , respectively.

Precision was diminished on the 1st postimmersion day: errors and variability of reproduction exceeded baseline levels considerably. Decline of accuracy of reproduction of exertion and position was similar and manifested in both instances more markedly in movements performed by the posterior group of crural muscles: magnitude of error constituted 24 and 28%, respectively, and in movements of dorsiflexion 17 and 15%; variability of reproduction constituted 15 and 24% in the former case, 10 and 14% in the latter. Less marked precision changes in dorsiflexion was also manifested by a lower level of significance of the demonstrated differences.

Conversely, task performance time with regard to displacement movements changed to the same extent in both directions, constituting almost double the base values (see Figure 2).

The depth of precision disturbances varied appreciably in different subjects. As can be seen in Figure 3, after immersion errors of reproduction of exertion ranged from +50 to -30% and variability from 5 to 40% in different subjects.

Individual analysis of tactics in performing motor tasks (Figure 4, I) revealed two main types of changes: in some subjects, movements became distinctly hypermetric, and magnitude of error increased with each successive movement (Figure 4, Ib); in others, the direction of error varied from movement to

movement (Figure 4, Ic). Interestingly, there was prevalence of the first type of error in plantar flexion, which constituted 67% for the group with respect to reproduction of exertion and 73% for reproduction of positions. In the group of movements for dorsiflexion, deviations in both directions were present in about the same number of cases: 44 and 55% with reproduction of exertion and 45 and 36% with reproduction of position.

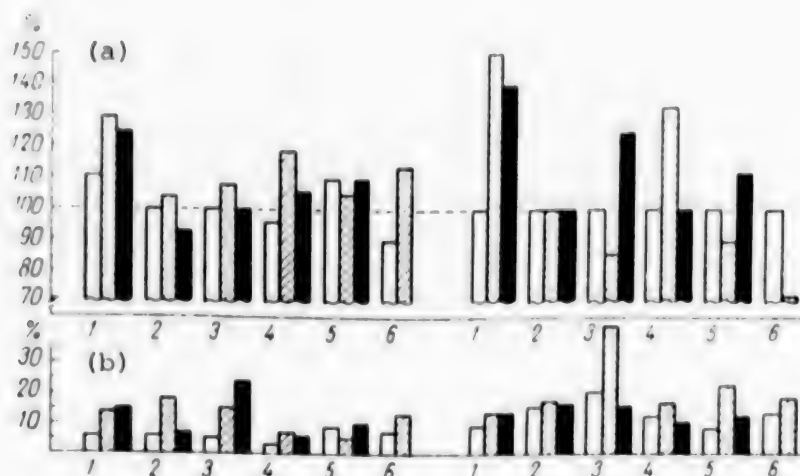


Figure 3. Reproduction of specified exertions before and after immersion hypokinesia by 6 subjects

Left, plantar flexion; right, dorsiflexion of foot. White bars--before immersion, striped and black--1st and 3d postimmersion days, respectively. Arabic numerals along x-axes refer to subject number.

- a) magnitude of reproduction (% of given, taken as 100%, dash line)  
b) coefficient of variation of reproduction (%)

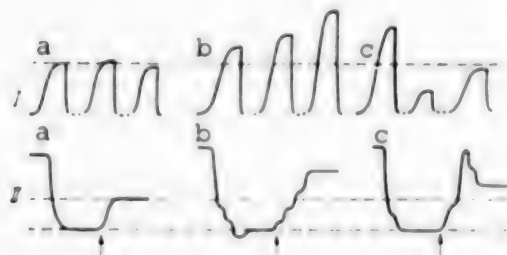


Figure 4.

Precision of movement (plantar flexion) before and after immersion hypokinesia. Dash lines--specified levels; arrows (at the bottom)--start of movement from 90° position

- I) reproduction of exertion  
II) reproduction of angular displacement  
a) baseline period  
b, c) types of postimmersion changes

Immersion also altered the structure of movements. As can be seen in Figure 4, II, the subjects performed displacement motions smoothly and rapidly before immersion. Their characteristics were consistent with those of rapid, programmed movements. Upon termination of immersion, the movements lost their inherent programmed speed and smoothness and became approximated, slow and fragmentary in bringing the foot to its target (Figure 4, IIb). When rapid tactics were retained (Figure 4, IIc), precision of movements was substantially diminished and the end position was reached through several corrections. Apparently, these changes in tactics

of finding the end position were the cause of the above-mentioned increase in time of performance of motor tasks.

The precision characteristics of movement presented a distinct tendency toward recovery by the 3d day of the recovery period. As can be seen in Figure 3, a significant increase in errors and variability of reproduction of exertion was found in only 2 subjects at this time, and in the others the characteristics of movements were close to baseline values.

These findings confirmed the hypothesis that impairment of movement precision is a consistent consequence of immersion hypokinesia. In our tests, these changes pertained to both precision of reproduction of muscular exertions and reproduction of positions. In other words, there is some decline of precision capacities of regulatory mechanisms. The extent of this decline is rather marked (particularly in plantar flexion situations) and it constitutes a mean of 24% for movements to reproduce effort and 28%, for positions. Precision of reproduction of movements for dorsiflexion diminished to a lesser extent: magnitude of change in this category of movements constituted a mean of 17% for reproduction of exertion and 15% for position.

The changes demonstrated in the structure of precision movements indicate that, without a static load, there was impairment in both the control circuit with feedback and in the programmed control circuit. In the former case, movements were no longer stereotypic, they usually became superfluous and hypermetric; in the latter case, they were either hypermetric or hypometric. In general, the changes in precision characteristics of movements were of the nature seen in ataxia.

With reference to the possible cause of atactic disturbances under these conditions, it can be related, first of all, to the muscular atonia that is present without a static load. As was demonstrated previously [1], immersion is associated with marked decline of muscle tone, which develops already on the 1st day of immersion and is consistently related to decrease in static load which is, as we know, a trigger for tonic reactions [10]. This hypothesis is confirmed by the fact that the precision changes in our tests were more marked for movements of plantar flexion than dorsiflexion. And, as it was shown, decline of muscle tone was noted primarily in crural extensors, which effect plantar flexion of the foot. But precision disturbances were present not only in isometric movements, but isotonic ones. This warrants the assumption that such disturbances may be due not only to reflex tonic changes, but central ones. In view of the foregoing, it is interesting to note that, in virtually half the cases, the magnitude of precision changes in different types of movement (exertion and displacement) in different directions (dorsal and plantar flexion) was correlated in the same subject, which could also be indicative of some common central mechanisms for their development. The presence of changes in the central parts of the motor system during immersion hypokinesia had been demonstrated in studies of excitability of spinal mechanisms according to parameters of T and H reflexes [4].

A comparison of the demonstrated disturbances to changes in precision control of efforts after 7 days of real weightlessness [4] revealed that, on the whole, the direction and magnitude of changes caused by removal of static load in

characteristics of the tested precision movements were very similar to those observed after short-term weightlessness, which also warrants the assumption that the mechanisms of their development are similar.

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EFFECT OF 'DRY' IMMERSION MODEL ON PARAMETERS OF FLUID-ELECTROLYTE METABOLISM, BLOOD PLASMA ALDOSTERONE AND CORTISOL LEVELS IN INDIVIDUALS DIFFERING IN BODY FLUID CONTENT

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[Article by M. A. Yunusov, V. N. Orlov and T. V. Vinokhodova]

[English abstract from source] Experiments were carried out to study the effect of dry immersion on fluid-electrolyte metabolism, aldosterone and cortisol content in 22 test subjects with varying content of water in the organism. It was found that dry immersion produced a diuretic effect and facilitated a higher excretion of electrolytes and a lower aldosterone content. The test subjects with a higher content of water in the organism (16 subjects) exhibited a more distinct and prolonged polyuria. During the recovery period the aldosterone content increased insignificantly and failed to return to the baseline level, in contrast to the subjects with a normal content of water in the organism.

[Text] It is known that man's change from customary gravity to weightlessness is associated with polyuria at the first stage of adaptation to the new environment. It is also believed that dehydration, with decline of circulating blood volume, is the secondary link in the chain of changes in homeostasis in weightlessness [2]. At the same time, the authors view redistribution of fluid with efflux from the lower body in a cranial direction as the initial triggering mechanism. Stimulation of volumoreceptors in the ostia of the venae cavae elicits reflex reactions (Henry-Gauer reflex), which lead to change in neuroendocrine regulation of fluid-electrolyte metabolism. Thus, with simulation of weightlessness by means of immersion in water, it was established that there is a drop in levels of antidiuretic pituitary hormone and aldosterone which, in the authors' opinion [9], causes polyuria and increased renal excretion of electrolytes. These homeostatic changes (hypovolemia, electrolyte deficiency) could affect appreciably the body's resistance in the recovery period and particularly in the first minutes of change in gravity. For this reason, the problem related to "painless" readaptation of man to change in gravity is not only of theoretical, but practical importance. We report here on a study of some parameters of fluid-electrolyte metabolism and neurohumoral components of blood (aldosterone, cortisol), which are involved in its regulation in individuals differing in body fluid content submitted to "dry" immersion.

## Methods

In this study we used the model of "dry" immersion proposed by Ye. B. Shul'zhenko and I. F. Vil'-Vil'yams [7]. We tested the effect of dry immersion on fluid-mineral metabolism (diuresis, excretion of Na and K in urine), plasma aldosterone and cortisol levels in 22 subjects. Electrolytes were examined on a flame photometer, aldosterone and cortisol levels, by a radioimmunological method with the CIS International kits of reagents. All of the subjects were on a standardized diet with intake of no more than 1200 ml fluid per day. The 1st group consisted of 12 essentially healthy men 30 to 55 years old with the usual body fluid content (no edema), who were exposed to dry immersion for 7 days. The 2d group consisted of subjects with marked (excessive) fluid retention or edema syndrome. All of the subjects in the 2d group (10 with ischemic heart disease, essential hypertension, cirrhosis of the liver) were submitted to 4-6-h sessions of dry immersion in our modification for therapeutic purposes [6]. Since the 2d group of subjects also received combined therapy (cardiac glycosides, diuretics and hypotensive agents), we used a standardized control period of 5 days, during which the same agents were used in identical doses and intervals on all subjects, in order to rule out the effect of this treatment on test results. We adhered to analogous conditions for 5 days after immersion.

Parameters of diuresis, Na, K in urine of subjects in the 1st group were assayed in the baseline period and for the first 3 days of immersion. In this group, we measured cortisol and aldosterone in the baseline period, after 15 min, 3, 7 h and 7 days of immersion (immediately and 1 day after its termination).

In the 2d group, parameters of fluid-electrolyte metabolism (daily diuresis, Na and K in urine) were measured in the control period, on the day of immersion and the next 2 days after it. Blood was drawn for aldosterone assay before immersion, after 2-3, 4-5 h of immersion and 20 h after the session of dry immersion.

The obtained data were processed by the method of variation statistics.

## Results and Discussion

We demonstrated marked polyuria with dry immersion, in subjects with both normal and high fluid content. It should be noted that analysis of the results for the 1st group of subjects revealed that maximum changes in parameters of diuresis and excretion of electrolytes in urine were observed on the first 2-3 days of immersion (Table 1).

Table 1 shows that there was maximum renal excretion of fluid on the first 2 days of immersion, which confirms the information in the literature on this score [12]. In this same period, we observed maximum excretion of Na and K in urine. Considering the 1.5-2-fold increase in Na/K ratio on the 1st-2d day of immersion, we can assume that the aldosterone mechanism is involved in the change in fluid-electrolyte balance [3]. However, some authors related the increase in diuresis during immersion to change in hemodynamics when simulating weightlessness which, in turn, leads to increased renal excretion of fluid and electrolytes [13].

Table 1. Dynamics of daily diuresis and electrolyte excretion on first 3 days of immersion in subjects with normal body fluid content (n = 6)

Parameter	Baseline	Day of immersion		
		1	2	3
Diuresis, ml/min	0.77 ± 0.02	1.24 ± 0.14***	1.22 ± 0.1**	0.86 ± 0.09
Na, meq/min	160.1 ± 12.3	255.0 ± 24.3***	245.0 ± 24.2**	175.0 ± 22.5
K, meq/min	46.05 ± 3.1	51.5 ± 5.6	57.2 ± 6.45	49.2 ± 1.7

Note: Here and in Tables 2 and 3, \*P<0.001, \*\*P<0.01, \*\*\*P<0.02, \*\*\*\*P<0.05 (in relation to baseline; reliability of differences was determined by Student's criterion obtained by the method of related pair variations [1]).

Table 2. Dynamics of plasma aldosterone and cortisol levels in subjects submitted to dry immersion for 7 days (n = 6)

Parameter	Baseline	Immersion				Recovery period
		15 min	3 h	7 h	7 days	
Aldosterone, ng/ml	119.6 ± 8.9	113.0 ± 6.8	80.4*** ± 11.4	83.6** ± 11.0	100.2 ± 12.9	125.3 ± 14.0
Cortisol, ng/ml	117.7 ± 14.9	133.67 ± 15.3	77.95 ± 11.5	94.15 ± 18.5	142.3** ± 14.4	127.5 ± 6.5

Table 2 lists the dynamics of plasma aldosterone and cortisol levels in the 1st group of subjects during 7-day dry immersion.

We see from the data in Table 2 that baseline aldosterone level did not differ appreciably from the normal parameters of the CIS International firm (in supine position, aldosterone level ranges from 25 to 125 ng/ml). We demonstrated a decline of aldosterone concentration by 32% after 3 h of immersion, as compared to the base period. This parameter was 30% lower than the baseline after 7 h. Assay of aldosterone level on the 7th day of immersion revealed a tendency toward approximation of the baseline value. However, aldosterone level reached baseline values only in the recovery period.

Our findings are consistent with the results of other authors, who also observed a decline of aldosterone level during water immersion starting with the first hours of submersion, as well as decreased excretion thereof in urine [8, 11]. However, these authors found a more marked decline of aldosterone than in our cases. When comparing the results, it must be borne in mind that the above-mentioned studies involved subjects sitting in water, and it can be assumed that under such conditions there is more marked hydrostatic pressure of water applied to the submerged parts of the body.

Examination of cortisol concentration revealed a tendency toward rise of its level by 14% after 15 min of immersion. As can be seen in Table 2, there was an insignificant decline of plasma cortisol level after 3 and 7 h of immersion

and reliable increase (by 32%) in its concentration on the 7th day of immersion. The literature concerning change in cortisol level during immersion is sparse and contradictory. Evidently, the tendency toward rise in cortisol level in the first hours of immersion is related to the fact that this hormone is one of the stressor substances. It would have been logical to assume that subsequent decline of cortisol during immersion could characterize conditions that are typical of rest and hypodynamia. There are also reports in the literature of the potentiating effect of cortisol on glomerular filtration. However, we were unable to detect a rise of its level in the period of maximum diuresis among subjects of the 1st group. As for elevation of cortisol level, along with aldosterone, by the 7th day of immersion, this tendency is of compensatory significance in the presence of hypovolemia, increased blood viscosity and electrolyte disturbances that are present at this stage of immersion [4]. At the present time, we know not only of the permissive effect of cortisol, but its property to cause mobilization of sodium from tissues and elimination of shortage of this electrolyte in blood [5].

The results of testing renal excretion of fluid and electrolytes in the 2d group of subjects, who were submitted to brief dry immersion, are listed in Table 3.

Table 3. Dynamics of diuresis and excretion of electrolytes in subjects with high body fluid content (edema) submitted to 4-6-h dry immersion (n = 10)

Parameter	Baseline	Day of immersion	Residual immersion reaction	
			2d day	3d day
Diuresis, ml	1536 ± 128	2630 ± 293	2076 ± 310	1865 ± 300
ml/min	1.0 ± 0.09	1.8 ± 0.6*	1.5 ± 0.8*	1.4 ± 0.7*
Na, meq/min	120.4 ± 9.2	173.5 ± 12.3*	160.0 ± 15.5*	130.0 ± 13.0
K, meq/min	37.2 ± 4.5	43.1 ± 5.6*	39.6 ± 3.4*	35.0 ± 3.11
K/Na ratio	3.5 ± 0.3	4.6 ± 0.4*	4.0 ± 0.41	3.7 ± 0.5

We see from the data listed in Table 3 that there was a marked increase in diuresis and electrolyte excretion, both on the day of 4-6-h immersion and on subsequent days. The most distinct residual reaction of diuretic effect of immersion was manifested on the first 2 days. We found that one session of immersion had an enhancing effect on diuretic therapy administered to these patients. Base plasma aldosterone level ( $189.7 \pm 20.1$  ng/ml) in subjects with high fluid content was considerably above normal (secondary aldosteronism). After 2-3 h of immersion, their aldosterone level dropped to  $127.2 \pm 15.5$  ng/ml ( $P < 0.05$ ) and after 4-5 h, to  $119.3 \pm 13.5$  ng/ml ( $P < 0.05$ ), i.e., by 33 and 37%, respectively, in relation to the baseline period. Aldosterone level remained 17% lower ( $156.9 \pm 10.6$  ng/ml;  $P < 0.05$ ) than the baseline 20 h after termination of brief immersion, which could be attributed, to the mechanism underlying the diuretic "aftereffect" of immersion. Fluid-electrolyte changes disappear immediately after immersion in subjects with normal body fluid content, and the aldosterone level reverts to baseline values within the first few hours of the recovery period [10].



From the standpoint of adaptation to immersion, we must mention the presence of changes in one direction (increased renal excretion of water, electrolytes, drop of aldosterone level) in both the 1st and 2d groups of subjects. Residual diuresis after immersion in subjects with high body fluid content could be indicative of inertness of regulatory mechanisms of fluid-electrolyte metabolism when changing to higher gravity. But they reacted just like essentially healthy individuals in a suspended state in fluid. The residual diuretic reaction after immersion in the subjects with high body fluid content may indicate that hemodynamic factors (which have an effect during immersion) do not play a part in eliminating fluid and electrolytes from the body.

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INTENSITY OF LIPID PEROXIDATION IN HYPOKINETIC RAT TISSUES

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[Article by T. Ye. Shidlovskaya]

[English abstract from source] The intensity of lipid peroxidation in the homogenates and mitochondria of the liver, heart and skeletal muscle of hypokinetic rats was measured. The primary products of lipid peroxidation, i.e., diene conjugates, were accumulated in all tissues on hypokinesia days 3, 15 and 30. The content of the final product--malonic dialdehyde--in the mitochondria increased on hypokinesia days 15 and 30. The low level of NADPH--and ascorbate-dependent lipid peroxidation in the mitochondria at early stages of hypokinesia (up to 15 days) and diene conjugates in homogenates on hypokinesia day 7 can be attributed to an activation of the protective systems of the organism against the immobilization stress. It is suggested that at early stages of hypokinesia the process of lipid peroxidation, or to be more precise lipid hydroperoxidation can be blocked.

[Text] Hypokinesia is an inevitable factor in many modern occupations (operators, pilots, cosmonauts and others). Investigation of the effects of hypokinesia permits simulation on earth of several of the effects of weightlessness. Under hypokinetic conditions one observes appreciable functional disturbances and changes in metabolism [6, 14], including lipid metabolism. The change in proportion of different groups of lipids, as well as between lipids and proteins, affects the structure of biological membranes, their permeability and integrity of organelles [2, 8]. Lipid peroxidation (LPO) processes play an important part in the mechanism of membrane alteration [3]. It has been proven that intensification of LPO is a typical reaction to extreme factors [7, 13]. At the same time, the role of LPO in onset and development of hypokinetic disturbances has been studied very little as yet [4, 5, 15].

We report here the results of a study of intensity of LPO in homogenates and mitochondria of the liver, heart and skeletal muscle of rats at different stages of hypokinesia.

## Methods

The studies were conducted on 51 control and 65 experimental male albino rats with initial weight of 170-200 g. Their movements were restricted by placing them in individual plexiglas box-cages. The animals were decapitated on the 3d, 7th, 15th and 30th experimental days. We took liver, heart and skeletal muscle tissue for examination. The mitochondrial fraction was recovered by differential centrifugation by the method of Garland [17] in a cold room at 0-4°C. The isolation medium contained 0.025 M tris-buffer in 0.175 M KCl, pH 7.4. The mitochondrial preparation was stored frozen. Intensity of LPO was assessed by the amount of diene conjugates in tissue homogenates and accumulation of malonic dialdehyde (MDA) in mitochondria during aerobic incubation in the presence of NADP·H or ascorbic acid. The levels of diene conjugates in homogenates were measured by a modification [3] of the Placer method. MDA was measured using the color reaction with thiobarbituric acid [16]. For examination of NADP·H-dependent LPO, the incubation medium contained 0.025 M tris-buffer, pH 7.4, 0.1 mM  $\text{Fe}^{2+}$ , 0.02 mM  $\text{Na}_4\text{P}_2\text{O}_7$ , 0.5 mM NADP·H 0.5-0.8 mg mitochondrial protein. To examine ascorbate-dependent LPO, NADP·H was replaced with 0.8 mM ascorbic acid, while the concentration of  $\text{Fe}^{2+}$  ions was reduced to 0.01 mM. Incubation at 37°C lasted 15 min. Proteins were assayed by the Lowry method and lipids, according to Bragdon as described by A. A. Pokrovskiy et al. [11].

## Results and Discussion

The Table lists data on LPO activity in liver homogenate and mitochondria. Accumulation of products of lipid peroxidation--diene conjugates--was already observed at the early stages of hypokinesia. Their level was 20% higher than in the control ( $P<0.05$ ) on the 3d experimental day, which is apparently related to onset of a stress situation due to immobilization. The findings of a number of authors indicate that activation of LPO processes is an inseparable component of metabolic changes associated with expression of the stress reaction [9]. On the 7th day, lipid hydroperoxides decreased in the liver homogenate to the control level, whereas on the 15th and 30th days they rose again by 53 and 29% ( $P<0.05$ ). Examination of ascorbate- and NADP·H-dependent LPO in liver mitochondria failed to demonstrate changes at the early stages of hypokinesia, whereas on the 15th and 30th days there was a tendency toward rise of MDA level.

Changes in LPO activity in the heart and skeletal muscle presented the same orientation as in the liver. In the heart (see Table), diene conjugate levels in a homogenate exceeded the control by 25, 10 and 45% ( $P<0.05$ ) on the 3d, 15th and 30th days, respectively. Unlike hepatic tissue, myocardial mitochondria presented intensive accumulation of MDA already on the 7th and 15th experimental days. When mitochondria were incubated with NADP·H, their MDA content increased by 30% ( $P<0.05$ ) on the 7th day and 18% ( $P<0.2$ ) on the 15th and 30th days. With incubation with ascorbic acid, mitochondrial MDA level was 35% higher than in the control on the 15th day ( $P<0.05$ ) and 17% higher ( $P<0.1$ ) on the 30th day.

Metabolic deviations, which occur with hypokinesia, develop earlier in skeletal muscles and are more marked [6]. In our experiments, the skeletal muscle homogenate presented maximum changes in intensity of LPO (see Table). At all stages of hypokinesia, diene conjugate levels were elevated and exceeded the control

by 77% ( $P<0.01$ ) by the 30th day. MDA in mitochondria increased by 28% ( $P<0.05$ ) in the case of ascorbate-dependent LPO and by 25% ( $P<0.1$ ) with NADP·H-dependent LPO, which coincides with data in the literature [15].

Levels of LPO products in hypokinetic rat liver, myocardium and muscle

Parameter studied	Animal group	Day of hypokinesia			
		3	7	15	30
Liver					
Diene conjugates in tissue homogenate, nmol/mg lipids	Control	4.12 ± 0.33 (6)	6.9 ± 0.49 (6)	4.32 ± 0.62 (9)	6.54 ± 0.74 (6)
	Experiment	3.29 ± 0.21 (11)*	3.1 ± 0.38 (8)	6.65 ± 0.34 (11)*	8.11 ± 0.29 (6)
MDA in mitochondrial fraction of ascorbate dependent LPO, nmol/mg protein	Control	2.92 ± 0.32 (6)	3.16 ± 0.41 (6)	3.60 ± 0.44 (6)	2.06 ± 0.33 (6)
	Experiment	2.86 ± 0.10 (6)	3.1 ± 0.28 (6)	3.24 ± 0.28 (6)	2.49 ± 0.13 (6)
Same for NADP·H-dependent LPO, nmol/mg protein	Control	2.54 ± 0.20 (6)	1.6 ± 0.18 (6)	1.83 ± 0.17 (6)	1.65 ± 0.14 (6)
	Experiment	2.02 ± 0.13 (6)	1.51 ± 0.13 (6)	1.81 ± 0.21 (6)	2.01 ± 0.19 (6)
Myocardium					
Diene conjugates in tissue homogenate, nmol/mg lipids	Control	4.81 ± 0.45 (6)	6.81 ± 0.43 (6)	8.20 ± 0.64 (6)	7.38 ± 0.71 (6)
	Experiment	5.99 ± 0.32 (11)*	6.76 ± 0.43 (6)	9.03 ± 0.69 (7)*	10.70 ± 0.11 (6)*
MDA in mitochondrial fraction of ascorbate dependent LPO, nmol/mg protein	Control	3.44 ± 0.08 (6)	3.22 ± 0.27 (6)	3.32 ± 0.41 (6)	1.76 ± 0.15 (6)
	Experiment	3.62 ± 0.20 (6)	3.31 ± 0.24 (6)	4.48 ± 0.21 (6)*	1.98 ± 0.02 (6)
Same for NADP·H-dependent LPO, nmol/mg protein	Control	2.05 ± 0.08 (6)	0.97 ± 0.076 (6)	0.560 ± 0.067 (6)	0.443 ± 0.036 (6)
	Experiment	2.20 ± 0.12 (6)	1.26 ± 0.136 (6)*	0.664 ± 0.063 (6)	0.520 ± 0.031 (6)
Skeletal muscle					
Diene conjugates in tissue homogenate, nmol/mg lipids	Control	4.29 ± 0.27 (7)	5.8 ± 0.52 (5)	5.11 ± 0.39 (10)	5.42 ± 0.51 (6)
	Experiment	6.22 ± 0.17 (11)*	7.56 ± 0.61 (7)	8.38 ± 0.69 (13)*	9.60 ± 0.73 (8)*
MDA in mitochondrial fraction of ascorbate dependent LPO, nmol/mg protein	Control	3.18 ± 0.25 (6)	3.06 ± 0.42 (6)	3.23 ± 0.29 (6)	2.68 ± 0.33 (6)
	Experiment	3.61 ± 0.38 (6)	3.12 ± 0.36 (6)	3.22 ± 0.31 (6)	3.44 ± 0.03 (6)*
Same for NADP·H-dependent LPO, nmol/mg protein	Control	1.96 ± 0.32 (6)	1.51 ± 0.11 (6)	0.696 ± 0.063 (6)	2.01 ± 0.15 (6)
	Experiment	2.07 ± 0.04 (6)	1.38 ± 0.11 (6)	0.632 ± 0.059 (6)	2.53 ± 0.24 (6)

Note: Number of animals given in parentheses; asterisks indicates  $P<0.05$ .

Our findings indicate that even relatively brief restriction of motor activity (up to 30 days) leads to significant changes in intensity of LPO. At the early stages, this is related to the effect of hypokinesia as a stress factor. However, there are special defense systems in the body that control processes of initiation of LPO and detoxification of peroxidation products. Their activation could explain the decline of diene conjugate level in homogenates of all tested tissues on the 7th day of our experiment. Absence of changes in extent of accumulation of MDA in mitochondria at the early stages of hypokinesia leads us to assume that there is an LPO block at the stage of formation of lipid hydroperoxides. This hypothesis is confirmed by the results of a study of activity of antioxidant enzymes under immobilization stress [10].



At the same time, protective systems are apparently not strong enough when hypokinesia lasts for a longer time. On the 15th and 30th experimental days, there was elevation of diene conjugate levels in homogenates and greater accumulation of MDA in rat tissue mitochondria. The metabolic disturbances inherent in hypokinesia provide favorable conditions for intensification of LPO. The change in tissue oxygenation at the early stage of immobilization causes more active utilization of oxygen as a prooxidant [1], while the changes in proportion of different groups of tissue lipids [12] lead to impairment of structural integrity of membranes. For this reason, lipids are more accessible to oxidation via the peroxide pathway. In turn, the decrease in share of phospholipids in tissues during long-term hypokinesia could be attributed to their intensive utilization in the LPO process.

The results of this investigation revealed that hypokinesia leads to significant changes in intensity of LPO. It can be assumed that these changes affect development of the hypokinetic syndrome, exerting an influence on membranes. This is associated with impairment of ion equilibrium, membrane permeability for metabolic products and regulatory factors [18, 19]. All this is indicative of the desirability of normalizing LPO under hypokinetic conditions in order to prevent and treat its sequelae.

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EFFECT OF 24,25-DIHYDROXYVITAMIN D<sub>3</sub> ON OSTEOGENETIC PRECURSOR CELLS IN  
IMMOBILIZED RATS

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[Article by V. N. Shvets, T. Ye. Burkovskaya, Z. Ye. Vnukova and O. Ye.  
Kabitskaya]

[English abstract from source] The experiments were carried out on Wistar SPF rats that were immobilized for 35 days. By heterotopic marrow cell transplantation under the kidney capsule to the normal rats and by cloning these cells in vitro it was found that osteogenetic potentials were significantly inhibited and the amount of osteogenetic precursor cells was reduced. The addition of 24,25(OH)<sub>2</sub>D<sub>3</sub> vitamin (at a dose of 1.25 µg per day) to the animal diet led to the normalization of the above parameters. It is assumed that immobilization-associated osteoporosis develops via, among other mechanisms, inhibition of histogenesis of stromal precursor cells. The beneficial role of vitamin D<sub>3</sub> is actually the activation of histogenesis of these cells which results in the recovery of bone remodelling during immobilization.

[Text] At the present time, we know of different causes of osteoporosis. However, its pathogenesis is still unclear, while the means of preventing and treating it are rather limited and have been little-studied. For the last 10 years, there has been intensive investigation of the role of vitamin D<sub>3</sub> and its active metabolites [12, 13].

In order to comprehend the mechanism of development of osteoporosis it is important to learn on which level of bone histogenesis changes arise: on the level of mature bone cells or cells that are precursors of osteogenesis.

Our objective here was to examine the osteogenetic potential of bone marrow and preventive effect of vitamin 24,25(OH)<sub>2</sub>D<sub>3</sub> on stromal precursor cells of rats deprived of static load on their skeleton. It is a known fact that man and animals develop immobilization osteoporosis under such conditions.

## Methods

In the experiments, we used 2-month-old Wistar SPF rats, which were kept for 35 days on a weighing stand [1], which removed the static load from hip bones and posterior extremities. The animals were divided into 4 groups: the 1st consisted of intact rats, the 2d of animals submitted to immobilization, the 3d of animals immobilized and given vitamin 24,25(OH)<sub>2</sub>D<sub>3</sub> and the 4th, intact animals given vitamin 24,25(OH)<sub>2</sub>D<sub>3</sub>. The rats were kept on a normal diet containing 0.6% calcium and 0.6% phosphorus, and they were given vitamin D<sub>3</sub> (synthetic form) by mouth in a dosage of 1.25 µg in 0.1% propylene glycol solution. After termination of the experiment, 2-5 rats from each group were decapitated, and bone marrow was extracted from their pelvic and tibial bones. Osteogenetic capacity of bone marrow cells was assessed by the method of heterotopic transplantation under the renal capsule and cloning in culture [6-8]. In the former case, tibial bone marrow was divided into equal fragments (about  $5 \cdot 10^6$  cells) and implanted under the renal capsule of a rat of the same line weighing 130-140 g. The kidneys were removed after 21 days, bone elements were extracted from underneath the capsule, they were weighed and imbedded in paraffin for subsequent histological and morphometric analysis. In the second variant, bone marrow was removed mechanically from pelvic bones, a suspension of cells was prepared and different doses (from  $0.6 \cdot 10^6$  to  $4 \cdot 10^6$  cells) were incubated at a temperature of 37°C in plastic vials (75 ml in size) in 10 ml medium 199 passed through 5% CO<sub>2</sub> with addition of 20% embryonic calf tissue and feeder ( $10^7$  guinea pig bone marrow cells exposed to radiation in a dosage of 50 Gy). We used 5-6 vials with each dose of cells. Exposure time for cells constituted 14 days without changing the medium.

## Results and Discussion

**Analysis of heterotopic transplantation.** The model of heterotopic transplantation of bone marrow cells under the renal capsule is convenient primarily because it permits evaluation of osteogenetic potential of hemopoietic cells according to their capacity to form bone tissue at the implantation site. Bone appears as a result of proliferation and differentiation of determinate cells, which are precursors of osteogenesis of donor origin, whereas the hemopoietic cells that settle in this bone are of recipient origin [5, 6]. Thus, with this experimental model one can investigate the capacities of cells that are precursors of osteogenesis when they are exposed to diverse extreme factors. Moreover, bone that develops under these conditions can be viewed as the springboard for hemopoiesis and a reservoir for mineral salts, which is rather important to the study of histogenetic relations between bone and hemopoietic tissue, as well as the mechanisms of impairment of mineral metabolism. On the basis of the foregoing, it can be assumed that this model is adequate enough for evaluation of functional properties of osteogenic precursor cells.

Table 1 lists the results of bone growth under the renal capsule in all 22 recipients tested (5-6 animals per group). As we see, there is appreciable change in volume of bone as a function of experimental conditions. The osteogenetic capacity of bone marrow cells of rats deprived of a static load on the skeleton diminishes with statistical reliability to about 1/5th, as compared to the intact control (bone weight is 1.5 mg, versus 4.12 mg in the control). Addition of vitamin D<sub>3</sub> corrects these changes, since the capacity of bone marrow cells of experimental rats to form bone is restored to the control level (see Table 1).



Table 1.  
Parameters characterizing bone development under rat kidney capsule ( $M \pm m$ )

Animal group	Bone weight, mg	Relative trabecular volume, %
1	$4.12 \pm 1.0$	$79.0 \pm 3.0$
2	$1.5 \pm 0.7$	$37.0 \pm 1.8$
3	$4.16 \pm 1.0$	$62.3 \pm 2.4$
4	$4.2 \pm 2.1$	$81.8 \pm 4.0$

Histological analysis of heterotopic bone revealed in all instances the presence of spongy bone, between the trabeculae of which there are bone marrow cells or, in their absence, delicate and friable connective tissue. Some of the bone elements (as a rule those not inhabited by hemopoietic cells) do not have osteoblasts and apparently reflect a regressing osteogenetic process and for this reason are not a satisfactory springboard for hemopoiesis. Such bone is encountered the most often after implantation of bone marrow from immobilized animals. Morphometric analysis

of relative volume of trabecular bone is submitted in Table 1, which shows that bone marrow from immobilized rats forms thinner bone tissue than marrow from intact animals (the differences are statistically reliable). Addition to the diet of experimental animals of vitamin D<sub>3</sub> leads to increase in volume of spongy bone which, however, does not reach the control level (see Table 1). Vitamin D<sub>3</sub> has no effect on osteogenetic potentiality of bone marrow from intact animals (see Table 1).

Thus, analysis of data obtained with the model of heterotopic transplantation enables us to conclude that an insufficient static load on the skeleton causes inhibition of osteogenetic capacity of precursor cells. It can be assumed that this phenomenon is related to reduction in number and/or decline of proliferative activity of osteogenetic precursor cells. This has been confirmed in experiments involving in vitro cloning of these cells.

In vitro experiments. The in vitro cell cloning method is used extensively for analysis of hemopoietic properties of hemopoietic cells that are the clonogenic precursors of stromal mechanocytes and, in particular, precursor of bone cells [3, 5]. The principle of this method amounts to the fact that when explanting low concentrations of cells in a culture dish, discrete colonies of fibroblasts develop with the stromal properties inherent in the original hemopoietic tissue. There is evidence of the fact that bone marrow cells that form colonies have osteogenic properties and that each colony is formed from one cell, which is called the fibroblast colony-forming cell--CFC<sub>f</sub> [2, 5, 8]. Proceeding from the capabilities of the described method, a series of experiments was conducted for analysis of number of CFC<sub>f</sub> in rats. As a rule, incubation of bone marrow cells was associated with formation of fibroblast colonies, the number of which was a linear function of dosage of explanted cells in the range of  $0.6 \cdot 10^6$  to  $2 \cdot 10^6$ . With increase in cell dose, the number of colonies settled on a plateau (Figure 1). This shows that the number of colonies on the linear segment of the curve corresponds to the number of CFC<sub>f</sub>. We encountered colonies with two types of structural organization: compact and reticulate (friable). Such stacking of fibroblast-like cells in colonies had been previously described by A. Ya. Fridenshteyn who explanted bone marrow cells from other animal species, and it is attributable to differences between CFC<sub>f</sub> within the same population [5]. In addition to structural composition, the colonies also differed in

alkaline phosphatase activity--phosphatase-positive and phosphatase-negative. Analogous types of colonies are encountered in explantation of mouse bone marrow [5]. It is assumed that expressly the CFC<sub>f</sub> that yield phosphatase-positive colonies have osteogenic activity [5].

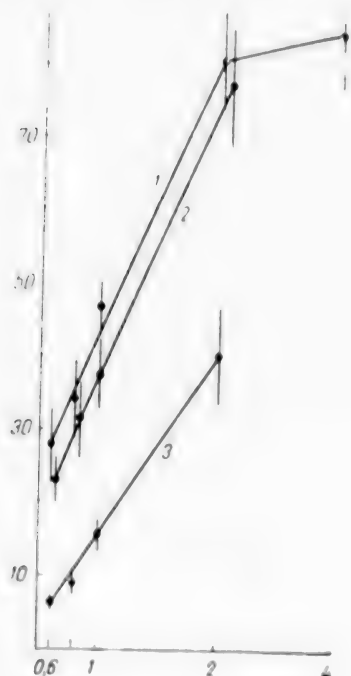


Figure 1.

Total number of fibroblast colonies as a linear function of dose of explanted cells from intact animals (1), rats given vitamin 24,25(OH)<sub>2</sub>D<sub>3</sub> after immobilization (2) and animals submitted only to immobilization (3)

Here and in Figure 2:

x-axis, number of cells  $\cdot 10^6$ .

y-axis, number of colonies ( $M \pm m$ )

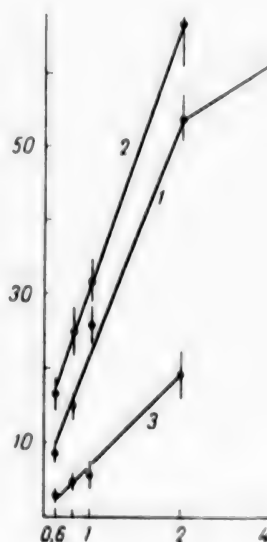


Figure 2.

Number of phosphatase-positive colonies as a linear function of dose of explanted cells

- 1) intact animals
- 2) rats submitted to immobilization + vitamin 24,25(OH)<sub>2</sub>D<sub>3</sub>
- 3) animals submitted only to immobilization

Figures 1 and 2 illustrate the results of quantitative analysis of colonies.

Thus, with incubation of different

volumes of bone marrow from intact rats,

effectiveness of production (total number) of phosphatase-positive and phosphatase-negative colonies constitutes 40-46.6 CFC<sub>f</sub> per  $10^6$  explanted cells. Efficiency of colony formation for both phosphatase-positive and phosphatase-negative fibroblasts drops to 1/2 to 1/4 with immobilization, and the proportion of these types of colonies fluctuates in the range of 40-60%, as in the control, with all doses of explanted cells. Daily addition to the diet of experimental rats of vitamin 24,25(OH)<sub>2</sub>D<sub>3</sub> led to restoration of efficiency of colony formation to the control level (37-39 CFC<sub>f</sub>/ $10^6$  explanted cells). Moreover, the efficiency of formation of phosphatase-positive colonies increased by 1.5-2 times, as compared to the control, and it constituted 80-90% of all colonies (see Figure 2). Measurement of colony size, which is indicative of extent of proliferative activity of CFC<sub>f</sub> offspring cells revealed that immobilization had a stimulating effect on growth of phosphatase-positive colonies with retention of phosphatase-negative ones at the level of normal size (Table 2).

Table 2. Mean size (mm) of different types of colonies (M±m)

Animal group	Phosphatase-positive colonies		Phosphatase-negative colonies		Number of colonies
	compact	reticulate	compact	reticulate	
1	2.67 ± 0.028	1.82 ± 0.007	2.23 ± 0.14	1.4 ± 0.006	1337
2	2.84 ± 0.05*	2.0 ± 0.035*	1.99 ± 0.046	1.45 ± 0.024	408
3	2.87 ± 0.025*	1.8 ± 0.015	2.18 ± 0.08	1.53 ± 0.028	731

\*Differences from control are statistically reliable.

Table 3.  
Proportion of colonies differing in structural stacking of cells

Animal group	Correlation between compact and reticulate colonies	
	phosphatase-positive	phosphatase-negative
1	1:3.7 (79)	1:14.5 (93.5)
2	1:1.3 (56)	1:2.2 (68)
3	1:1.7 (63)	1:3.7 (78.6)

Note: Number of reticulate colonies (as % of total number) is given in parentheses.

only insignificantly the level noted with immobilization alone (see Table 3).

Thus, the data on size and proportion of different types of colonies indicate that with reduction of static load on the skeleton both quantitative and qualitative changes occur within the population of stromal precursor cells. Vitamin D<sub>3</sub> corrects essentially the quantitative changes in the CFC<sub>f</sub> population.

To sum up the results of studies obtained by both methods, we can draw several conclusions: 1) when there is an insufficient static load on the skeleton, the osteogenetic capacity of bone marrow is diminished due to decrease in number of cells that are precursors of osteogenesis; 2) the decrease in number of CFC<sub>f</sub> with increase in rate of proliferation of remaining cells is apparently due to "forced" change of histogenesis of bone to a lower functional (compensatory) level; 3) vitamin 24,25(OH)<sub>2</sub>D<sub>3</sub> has a beneficial effect on the CFC<sub>f</sub> population and more so on phosphatase-positive CFC<sub>f</sub>, restoring their number to the control level or higher. This restoration should lead to faster bone histogenesis and "removal" of osteoporosis, which is what we had established previously. At the present time, there are data indicative of the direct effect of vitamin D<sub>3</sub> on bone. It was found that bone cells, mainly osteocytes, have specific receptors for vitamin D<sub>3</sub> [9, 10, 15] and can metabolize 25(OH)D<sub>3</sub> into 1,25(OH)<sub>2</sub>D<sub>3</sub> and 24,25(OH)<sub>2</sub>D<sub>3</sub> [11]. Results of experiments involving long-term immobilization of rats revealed that, under these conditions, there is decline of active

metabolites of vitamin D<sub>3</sub> in bones [4]. The cited data, together with the results of this study, warrant the assumption that osteogenesis precursor cells have membrane receptors for 24,25(OH)<sub>2</sub>D<sub>3</sub> and, consequently, are sensitive to changes in vitamin D<sub>3</sub> metabolism in the body and bone tissue. The second hypothesis ensuing from the results of this study is that inhibition of histogenesis of precursor cells is one of the pathogenetic mechanisms of immobilization-related osteoporosis. This is indicated by the low weight of heterotopic bone and its thinning, absence of osteoblasts and bone marrow in such bone, and significant decrease in number of stromal precursor cells. Perhaps all of the above is the cause of decline of the process of bone remodeling as a result of change, in the terms of Frost [14], in the "bone remodeling unit" and functional expression of its "bone metabolism unit" which have a direct bearing on mineral homeostasis. Apparently, the beneficial role of exogenous vitamin D<sub>3</sub>, which diminishes the degree of development of immobilization osteoporosis, consists of stimulating osteogenesis precursor cells, which is associated subsequently with restoration of activity of the bone remodeling and metabolic unit, the function of which had been inhibited due to the shortage of mechanical load.

Whether this mechanism affects the entire skeleton or prevails only in bones that carry a static load is not clear as yet, i.e., we are referring to the fact that if the entire population of osteogenesis precursor cells "suffers," osteoporosis may spread entirely over the bone system, regardless of support function of different parts of the skeleton. It is also still unclear if there is an analogous pattern of change in histogenesis on the level of precursor cells with development of other types of osteoporosis (senile, postclimacteric, dietetic, thyroid, glucocorticosteroid, osteoporosis in weightlessness, idiopathic, etc.). Investigation of all the above questions is a promising direction of research in order to elaborate tactical systems and means of prevention and treatment of osteoporosis of different etiology. As for vitamin D<sub>3</sub>, in particular, its active metabolite 24,25(OH)<sub>2</sub>D<sub>3</sub>, to assess its effect as a whole, it can be concluded that vitamin D<sub>3</sub> alone is not enough for complete correction of changes in osteogenesis due to immobilization. Apparently, it must be combined with other osteotropic, biologically active agents.

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BASIC RESULTS OF EXPERIMENTS WITH LONG-TERM ROTATION OF RATS AS APPLIED TO  
THE PROBLEM OF ARTIFICIAL GRAVITY

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19,  
No 4, Jul-Aug 85 (manuscript received 4 May 84) pp 53-57

[Article by A. R. Kotovskaya, I. B. Krasnov and A. A. Shipov]

[English abstract from source] Rats housed as a group per cage were centrifuged for 21 and 30 days at 1.1 and 2.0 G. The following parameters were measured: motor activity, body mass, static and dynamic endurance, acceleration (+Gz) tolerance, vestibular function, equilibrium function, skeletal muscle contractility, bone dynamics, gas exchange, blood biochemistry, weight of adrenals, thymus and thyroid gland; morphology of adrenals, thyroid gland, and cortex of the cerebellum nodulus; biochemistry of blood hormones, energy metabolism enzymes in the liver, bone phosphatase, myosin Ca-Mg-ATPase in the myocardium, protein sulfhydryl groups in the cerebellar motor cortex. The study has demonstrated that prolonged (1/50 of their life time) centrifugation of unrestrained rats causes no deterioration of many physiological functions, i.e., rotation produces no adverse effects on the animal body.

[Text] The existing plans for large profitable settlements in space provide for the presence of artificial gravity (AG) in such areas [5]. Nor is the possibility ruled out that, in the future, AG generated by rotation of a spacecraft will be a means of precluding the effect of weightlessness on man during spaceflights [4].

Of course, the AG parameters for a manned flight must be determined through studies of humans. At the same time, animal experiments can solve such problems as validation of AG as a means of preventing the effect of weightlessness during spaceflights and evaluation of the significance of rotation factors to organisms on different levels of organization.

Experiments performed in 1962-1963 by A. A. Gyurdzhian et al. [3], as well as those conducted in preparation and implementation of the research program aboard Cosmos-936 biosatellite [2], demonstrated convincingly that the general direction and basic patterns of changes in motor acts, vestibulosomatic

reflexes, parameters of the body's physical state and other physiological functions during and after spending time in rotating systems can be studied with success on laboratory rats.

We shall discuss here the results of ground-based experiments, which were a continuation of the studies begun on *Cosmos-936* with AG on board.\*

The purpose was to investigate the distinctions of somatic reactions, as well as reactions of several gravity-dependent systems in mammals (rats), to long-term (21 and 30 days) rotation.

The experiments were conducted with free (nonimmobilized) groups of animals kept in centrifuge containers to rule out the influence on tested functions of artificial restriction of movement, as well as asymmetry of mechanical factors. In each experiment, there was a central group (1st) of animals used to assess the effect of the rotation factor on parameters studied (level of gravity at the point in the container most distant from the axis of rotation was 1.1 G) and peripheral group (2d), to assess the tested reactions as a function of magnitude of gravity (2.0 G).

In the experiments with 21-day rotation we examined motor activity, changes in body mass, static and dynamic endurance, resistance to accelerations (+Gz), vestibular function, equilibrium, biometry and contractility of skeletal muscles, peripheral blood, parameters of bone dynamics and total gas exchange.

In the 30-day rotation version, we examined changes in weight of the body, adrenals, thymus and thyroid; we conducted morphometric studies of the bone system, morphological studies of the adrenals, thyroid, structure of cerebellar nodulus, biochemical studies of blood hormones, activity of enzymes of energy metabolism in the liver, phosphatases of bone, Ca-Mg-ATPase of myosin in the myocardium, protein sulfhydryl group levels in the motor cortex.

The results of our experiments revealed that the animal's lost weight in the 1st week of rotation and showed increase in static and dynamic endurance (A. A. Shipov and L. A. Tabakova); there was inhibited growth in length and width of long bones (V. N. Shvets, O. Ye. Kabitskaya). There were typical changes in motor activity: it diminished for the first 2 days and increased drastically by the 7th day. Thereafter, these parameters gradually reverted to normal, and by the 15th-21st day of rotation did not differ from values inherent in the control group (3d) of animals. The changes were nonspecific, demonstrating the animals' stress reaction to unusual living conditions.

By the 21st day of rotation, there was increase in weight, force, work capacity and rate of development of contraction in antigravity muscles (V. S. Oganov, S. A. Skuratova); there was a tendency toward slower growth of the femur in length and increase in thickness of cortical layer of the diaphysis, activation of apposition growth and slowing of endosteal resorption (A. M. Baulin). These are specific reactions indicative of an increased functional load on the skeletomuscular system during rotation. In the course of rotation, there was

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\*The most important results will be reported in detail in different publications.

a tendency toward increased oxygen uptake and carbon dioxide output, decline of  $pO_2$  in tissues (V. L. Popkov), as well as toward increase in blood hemoglobin, hematocrit and neutrophil-leukocyte index (I. I. Britvan). We should also include in specific reactions, the decline during rotation in reactivity and sensitivity of the functional system of semicircular canals due to the periodic effect on the vestibular system of procession accelerations, which arose when the animals moved about (A. A. Shipov, L. A. Tabakova).

In the aftereffect period following 21-day rotation, there were no demonstrable differences in static and dynamic endurance, resistance to accelerations ( $+G_z$ ) and equilibrium function between parameters for animals in the 1st, 2d and 3d groups. On the 7th-11th days of the aftereffect period, parameters of peripheral blood, gas exchange and nystagmic reactions reached levels inherent in the 3d group of animals.

It is important to note that exposure of rats in unrestricted groups for 21 days to a rotating system at gravity levels of 1.1 and 2.0 G does not worsen equilibrium or physical condition according to static and dynamic endurance, tolerance to head-pelvis accelerations and contractility of muscles. The results indicate that some of the facts demonstrated during experiments aboard Cosmos-936 [2] with onboard AG (drastic decline of static endurance on the last day of the experiment, prolonged worsening of equilibrium, etc.) are largely related to conditions where animals were kept in small box-cages.

By the 30th day of rotation the 1st and 2d groups had, on the whole, adapted to unusual living conditions. This was indicated by such factors as absence of signs of a stress reaction (the animals showed no change in absolute or relative weight of adrenals and thymus, the subglomerular zone of the adrenal cortex presented normal structure [Ye. A. Savina]); there was some decrease in plasma adrenocorticotrophic hormone content (B. V. Afonin) and change in some systems for the purpose of their more intensive function at higher gravity. In particular, morphological studies revealed increase in functional activity of the thyroid and activation of its system of C cells, which was associated with release of secretions containing calcitonin (G. I. Plakuta-Plakutina). According to biochemical tests, there was reliable increase in plasma calcitonin content (B. V. Afonin), which confirms the demonstrated activation of C cells of the thyroid and is indicative of increased activity of this hormonal element of regulation of calcium metabolism in bones. There was increase in acid phosphatase activity in long bones, which was indicative of activation of osteoclasts and reorganization of bone tissue (I. A. Popova). Rotated animals exposed to 2.0 G gravity presented drastic increase in myocardial myosin Ca-Mg-ATPase which increased its contractility (Ye. A. Nosova). There was structural change in the granular layer of the cortex of the cerebellar nodulus, indicative of passage to these structures of increased impulsation from gravireceptors (I. B. Krasnov, I. I. Babichenko).

At the same time, the animals in the 1st and 2d groups revealed a decrease in activity of oxidative enzymes of the Krebs' cycle in hepatocytes, similar to the findings in the liver of hypokinetic rats (Ye. G. Vetrova). This can apparently serve as indirect confirmation of the report of diminished motor activity immediately after rotation, which was more marked in the 2d group of animals. This is also indicated by the tendency toward decline of protein



SH group content of the motor cortex as an indicator of diminished functional activity of the cerebral cortex (A. N. Panov, Ye. P. Shelepina).

After rotation for 30 days, the 2d group of animals presented a tendency toward retardation of growth, which was manifested by decreased increment of body weight (I. B. Krasnov). This group of rats showed activation of the renin-angiotensin-aldosterone (RAA) system (B. V. Afonin), generally similar to the findings in man exposed to brief accelerations [1], but differing in that there was insignificant increase in activity of renin, which is the triggering element of the RAA system.

Thus, 30-day rotation led to changes in most tested systems that were in the same direction in the 1st and 2d groups of animals, but they were more marked in the 2d group.

Removal from the rotating system was not associated with a stress reaction, since no morphological signs of stress were demonstrable in the adrenal cortex on the 2d and 7th days after 30-day rotation; weight of the adrenals and thymus did not differ from that of animals in the 3d group (Ye. A. Savina), while plasma adrenocorticotrophic hormone content remained low (B. V. Afonin).

On the 2d day of the aftereffect period following 30-day rotation, there was decline of several parameters characterizing the level of functional activity of the cardiovascular and bone systems, which experienced a higher functional load during rotation. Activity of myocardial myosin Ca-Mg-ATPase dropped to the control level (Ye. A. Nosova), there was less activation of the C cell system of the thyroid (G. I. Plakuta-Plakutina), decrease in calcitonin content of blood plasma (B. V. Afonin) and in activity of bone acid phosphatase (I. A. Popova). At the same time, there was even greater activation of the RAA system (B. V. Afonin). We should mention normalization of protein SH group levels in the cerebral cortex (A. N. Panov, Ye. P. Shelepina), which was indicative of restored functional activity of the motor cortex, as well as activity of oxidative enzymes in the liver that furnish energy for biosynthetic processes (Ye. G. Vetrova).

On the 7th day of the aftereffect period following 30-day rotation, most of the tested parameters of metabolism and structure of tissue and organs in animals of the 1st and 2d groups did not differ from control animals, and only the high level of plasma calcitonin (B. V. Afonin) and high activity of acid phosphatase in bone tissue (I. A. Popova) were indicative of continued reorganization of bone structure.

Thus, 30-day exposure of rats to a rotating system at close to 1.0 G, where they were kept in free groups, either failed to alter the tested physiological parameters or else the changes were in the form of mild tendencies. In animals rotated at 2.0 G, several systems (primarily the myocardium and skeletomuscular system) presented changes indicative of reorganization to provide for their more intensive function in the presence of an increased gravity load.

The results of these experiments enable us to draw the following conclusion: long-term (1/50th of lifetime) exposure to a rotating system where rats are

kept in unrestricted groups is not associated with worsening of physiological functions according to a wide set of parameters, i.e., under these conditions rotation factors do not have an adverse effect on animals.

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MAN'S TOLERANCE TO FULMINANT FORM OF HYPOXIC HYPOXIA

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 20 Jan 84) pp 57-60

[Article by A. Yu. Katkov, Ye. P. Vyazova, R. N. Chabdarova, I. S. Krikun and Zh. M. Kudryashova]

[Text] Rapidly progressing, or "fulminant" form of hypoxic hypoxia is among the extreme factors to which one is exposed in case of depressurization of a flight vehicle [10]. It can be simulated by means of breathing with inert gas. We submit here the results of testing reactions to fulminant hypoxia elicited by breathing nitrogen.

Methods

Determination was made of "reserve time" when breathing nitrogen in 45 subjects. This referred to the time from the start of the first inspiration of nitrogen to subject's loss of capacity to perform a writing test. During the tests, we continuously recorded the heart rate (HR) and pneumogram, electroencephalogram (EEG) in the occipital and frontal leads, minute volume (MV) and parameters of total gas exchange. The latter included CO<sub>2</sub> output and O<sub>2</sub> uptake with air breathing, and CO<sub>2</sub> and O<sub>2</sub> output with nitrogen breathing [3]. With the latter, we recorded concurrently oxygen tension in alveolar air (on a radio-frequency mass spectrometer) and in venous blood (using a blood gas analyzer). The latter was also used to determine pH and CO<sub>2</sub> tension in venous blood. A CO-oximeter 282 was used to determine oxygenation of venous blood. Venous blood was also used to assay lactate and pyruvate by the spectrophotometric method. Blood was drawn before nitrogen breathing, during the test and 5 min after it from a vein in the right arm after putting it in a splint (to eliminate convulsive muscular contractions which occur as a result of hypoxia). During nitrogen breathing, blood was taken from the vein at the final stage for 5 s, starting at the moment that the subject could no longer perform the writing test. Vessels of the fundus of the eye were examined to observe reactions of cerebral vessels to the fulminant form of hypoxia. A GEO-68 visuscope was used, with the conventional method, to measure the caliber of retinal arteries and veins before nitrogen breathing, in its terminal phase and 5 min after. Polarography was used to measure O<sub>2</sub> tension in forearm skin.

## Results and Discussion

In the course of determining the "reserve time" of nitrogen breathing, the subjects were divided into two groups. The 1st group consisted of individuals with "reserve time" of  $55 \pm 3.3$  s (individual fluctuations 50-60 s). The 2d group consisted of subjects with  $80 \pm 2.4$  s (individual fluctuations 70-95 s) "reserve time." At the moment they became unable to perform a writing test, all subjects presented an increase in slow EEG activity. The results of examining cardiorespiratory system reactions to fulminant hypoxia are listed in Table 1. This table shows that less marked reactions of pulmonary ventilation and  $O_2$  output were inherent in subjects with longer reserve time. This had also been observed previously with voluntary reduction of pulmonary ventilation during nitrogen breathing by means of special training, as well as in the case of diminished sensitivity of the respiratory center to hypoxemia in the presence of 2-week starvation [2, 3, 6, 7].

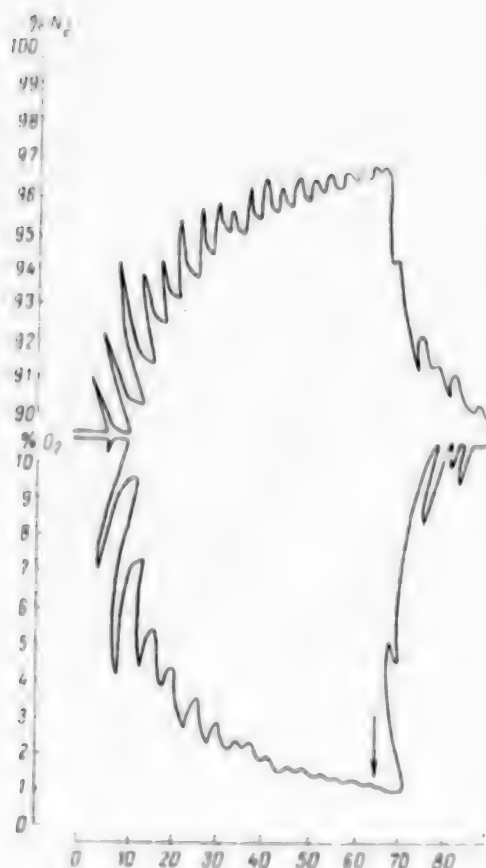
In view of the fact that, in the relatively short period of breathing nitrogen, oxygen uptake by tissues from its reserve in the body could apparently not have changed appreciably, we introduce the parameter of "degree of deoxygenation" (DD). We calculated DD by adding  $O_2$  uptake and  $O_2$  output within the indicated period then multiplying this sum by duration, in minutes, of nitrogen breathing. Mean DD for subjects with lesser and greater "reserve time" constituted 1062 and 1276 ml  $O_2$ , respectively. If we consider that the total oxygen in an adult man of average weight is about 2440 ml, we shall see that the body was deprived of about 44-52% of its oxygen reserve over the entire period of nitrogen breathing.

Table 1. Reaction of human cardiorespiratory system to nitrogen breathing as a function of reserve time.

Time of examination	Group	reserve time, s	HR/min	MV, l/min	$O_2$ uptake, ml/min	$O_2$ output, ml/min	$O_2$ output, ml/min
Before breathing nitrogen	1	$55 \pm 3.3$	$70 \pm 11$	$21 \pm 0.27$	$0.19 \pm 0.08$		$21 \pm 0.09$
	2	$80 \pm 2.4$	$71 \pm 11$	$22 \pm 0.27$	$0.18 \pm 0.07$		$22 \pm 0.14$
During N breathing	1	$55 \pm 3.3$	$101 \pm 11$	$16 \pm 0.14$		$186 \pm 59$	$145 \pm 40$
	2	$80 \pm 2.4$	$78 \pm 11$	$18 \pm 0.29$		$177 \pm 55$	$144 \pm 16$
						$P = 0.05$	$P = 0.001$

Table 2 lists the results of a comparative study of dynamics of oxygen tension in alveolar air ( $p_AO_2$ ), venous blood ( $p_vO_2$ ), forearm skin tissue ( $p_TO_2$ ), as well as venous blood oxygenation ( $Hb_vO_2$ ), which was conducted on 9 subjects. As can be seen in Table 2, at the moment that nitrogen breathing stopped  $p_AO_2$  was lower in all subjects than  $p_vO_2$ . After breathing nitrogen for 15 s,  $p_AO_2$  was lower than  $p_vO_2$  in only 1 subject (group mean  $44 \pm 3.1$  mm Hg), and after 30 s, already in 5 cases (mean  $32 \pm 3.2$  mm Hg). After 45 s of nitrogen breathing, group mean  $p_AO_2$  was  $25 \pm 2.5$  mm Hg, and it was lower than the end  $p_vO_2$  in 7 out of 9 subjects. The dynamics of replacement of oxygen with nitrogen in alveolar air, as recorded on the mass spectrogram, are illustrated in the Figure.





Dynamics of replacement of oxygen with nitrogen in alveolar air on radio-frequency mass spectrogram during nitrogen breathing

X-axis, time (s); arrow shows time of changing from nitrogen to air for breathing

like  $p_{VO_2}$ , it continued to decline due to oxygen debt. Arterial oxygenation, as determined by the oxyhemometric studies, decreased to 65-60% at the time nitrogen breathing was stopped and this was followed by rapid restoration of base values [1].

A comparative study of dynamics of  $p_{VO_2}$  during nitrogen breathing in the presence of oxygen debt, which developed after determination of tolerance to gradually progressive hypoxia with rarefaction of atmosphere in a pressure chamber [5], was made on 5 subjects. Total exposure to hypoxia in the chamber lasted  $45 \pm 0.8$  min, and the "ceiling" reached was at  $8600 \pm 250$  m. "Reserve time" of nitrogen breathing was  $75 \pm 10.5$  s before "ascent" in the chamber and  $65 \pm 13.9$  s 5-6 min after it. Before the pressure chamber "ascent,"  $p_{VO_2}$  during nitrogen breathing dropped from  $48 \pm 7.9$  to  $43 \pm 2.1$  mm Hg, and after the test it constituted  $44 \pm 7.7$  mm Hg. After termination of exposure to hypoxia in the pressure chamber,  $p_{VO_2}$  dropped to  $19 \pm 1.6$  mm Hg. Against this background, nitrogen breathing led to elevation of  $p_{VO_2}$  to  $25 \pm 1.6$  mm Hg ( $P < 0.05$ ), and this level persisted for 5 min after termination of the test ( $24 \pm 2.8$  mm Hg). This series

There was almost no change in  $p_{T}O_2$  for the first 15 s of nitrogen breathing, and it constituted a mean of  $35 \pm 1.0$  mm Hg. It was  $29 \pm 1.1$  mm Hg after 30 s of breathing nitrogen and  $24 \pm 1.5$  mm Hg after 45 s. The decline of  $p_{T}O_2$  in the skin is attributable not only to decrease in oxygen of blood but peripheral vascular spasm. In particular, it was established that there is a spasm of arterial ends of capillaries of the lower lip and nail bed of man already after 15 s of nitrogen breathing. This spasm extends to venous parts of the capillaries and progresses until there is complete emptying of microvessels [6, 7]. Due to spasm of peripheral microvessels, conditions develop for increasing delivery of blood to vital organs. For example, in our studies, at the last stage of nitrogen breathing there was drastic dilatation of retinal vessels. The caliber of the central artery increased from  $80.9 \pm 0.5$  to  $110.7 \pm 1.3$  nm and that of the central vein, from  $150.7 \pm 3.1$  to  $189.3 \pm 3.4$  nm. There was marked retinal hyperemia. The caliber of these vessels decreased 5 min after terminating nitrogen breathing, but did not reach base values. According to dynamics of REG, dilatation of cerebral vessels persists for about 10 min after stopping nitrogen breathing [4, 8].

The decline of  $Hb_{VO_2}$  during the period of nitrogen breathing turned out to be statistically unreliable, but after the test,

of studies graphically proves that there is deoxygenation of the body when breathing with nitrogen, when oxygen passes from tissues into blood. This view is also confirmed by the dynamics of  $HbVO_2$  during nitrogen breathing after "ascent" in pressure chamber. By the end of the period of nitrogen breathing, this parameter rose from  $30 \pm 3.8$  to  $43 \pm 4.8\%$  ( $P < 0.05$ ), and after 5 min of breathing with oxygen it rose to  $46 \pm 6.7\%$ . Analogous deoxygenation in the presence of extremely acute hypoxia had been observed previously in experiments with animals [9].

Table 2. Effect of breathing with nitrogen on oxygenation of human body

Subjects	Nitrogen breathing reserve time, s	Before N breathing				At final stage of N breathing				5 min after N breathing			
		$PAO_2$	$PAO_2$	$PO_2$	$HbVO_2$	$PAO_2$	$PAO_2$	$PO_2$	$HbVO_2$	$PAO_2$	$PAO_2$	$PO_2$	$HbVO_2$
		mm Hg				mm Hg				mm Hg			
10	0	110	110	38	30	110	110	38	30	110	110	38	30
	10	105	105	38	30	105	105	38	30	105	105	38	30
	20	100	100	38	30	100	100	38	30	100	100	38	30
	30	95	95	38	30	95	95	38	30	95	95	38	30
	40	90	90	38	30	90	90	38	30	90	90	38	30
	50	85	85	38	30	85	85	38	30	85	85	38	30
	60	80	80	38	30	80	80	38	30	80	80	38	30
	70	75	75	38	30	75	75	38	30	75	75	38	30
	80	70	70	38	30	70	70	38	30	70	70	38	30
	90	65	65	38	30	65	65	38	30	65	65	38	30
10	0	110	110	38	30	110	110	38	30	110	110	38	30
	10	105	105	38	30	105	105	38	30	105	105	38	30
	20	100	100	38	30	100	100	38	30	100	100	38	30
	30	95	95	38	30	95	95	38	30	95	95	38	30
	40	90	90	38	30	90	90	38	30	90	90	38	30
	50	85	85	38	30	85	85	38	30	85	85	38	30
	60	80	80	38	30	80	80	38	30	80	80	38	30
	70	75	75	38	30	75	75	38	30	75	75	38	30
	80	70	70	38	30	70	70	38	30	70	70	38	30
	90	65	65	38	30	65	65	38	30	65	65	38	30
		$70 \pm 1.8$				$70 \pm 1.8$				$70 \pm 1.8$			

Pyruvate content of venous blood did not change appreciably under the effect of nitrogen breathing, constituting  $0.89 \pm 0.19$  mg/100 ml before the test,  $0.79 \pm 0.16$  at the end of it and  $1.26 \pm 0.18$  5 min after termination of the test. Venous blood lactate content, however, increased during nitrogen breathing from  $26.15 \pm 0.77$  to  $28.85 \pm 0.38$  mg/100 ml, and  $20.38 \pm 0.27$  after the end of the test. Thus, there was intensification of the glycolysis process with nitrogen breathing. No appreciable changes were demonstrated in  $pH$  and  $CO_2$  tension of venous blood during nitrogen breathing. By the end of the nitrogen test, these parameters changed from  $7.37 \pm 0.016$  to  $7.38 \pm 0.021$  and from  $37 \pm 1.8$  to  $40 \pm 1.8$  mm Hg, respectively. They constituted  $7.44 \pm 0.022$  and  $37 \pm 2.5$  mm Hg 5 min after nitrogen breathing.

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# INVESTIGATION OF CATECHOLAMINE METABOLISM AT HIGH ALTITUDES

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 14 Oct 83) pp 60-63

[Article by N. A. Davydova, Yu. A. Senkevich, M. S. Belakovskiy and S. V. Samratova]

[English abstract from source] The function of the sympatho-adrenal system (SAS) measured with respect to the content of epinephrine and norepinephrine in blood and urine as well as dopamine and DOPA in urine of mountaineers and athletes non-trained to high altitudes was examined. It was found that the mediator component of the SAS was activated in the athletes and that the adaptive capability of the SAS of the mountaineers was adequately high. It is recommended to study the SAS activity as a diagnostic test for measuring the fitness of members of expeditions in an unusual environment.

[Text] The sympathoadrenal system (SAS), which has adaptive and trophic function, plays an important part in formation of adaptive reactions to altitude hypoxia. Several authors have shown that man's tolerance to hypoxia corresponding to an altitude of about 5000 m above sea level depends on his body's capacity to release norepinephrine (NE). These authors believe that hypoxia is the prime cause of increase in blood and urine NE content [11, 13].

Our objective here was to assess, by means of some parameters of catecholamine (CA) metabolism, the capacity of individuals differing in degree of conditioning to adapt to high altitudes. It is of practical importance to examine this matter, since the success of high-altitude expeditions, the life and health of their participants depend on their adaptability.

## Methods

Two groups of athletes of studied at high altitude. The 1st group consisted of 25 mountain climbers, who were candidates for an expedition to Everest. They were examined during the period of a training rally in Tyan-Shan mountains (January) at an altitude of 1700 m above sea level. Venous blood was drawn and 24-h urine collected 1 day before ascending Komsomol peak (4376 m above sea level) and on the day after the return.



Athletes without special training (13 men and 4 women) made up the 2d group. They were examined during the high-altitude passage of Medeo--Lake Issyk-Kul through Zailiyskiy Alatau (August); the altitude was up to 4000 m above sea level. Urine was collected daily as in the 1st group. Both groups were on a controlled diet.

SAS activity was assessed by the concentration of epinephrine (E), NE in blood plasma (1st group) and excretion of E, NE, dopamine (DA) and dopa in urine (1st and 2d groups) assayed by fluorimetric methods [12]. Qualitative evaluation of changes in activity of the hormonal and transmitter elements of SAS was made according to estimated parameters of relative CA metabolism activity [10]. The results of the tests were processed by the method of variation statistics using the Fisher-Student criterion [1].

### Results and Discussion

The results of our studies are listed in Tables 1 and 2. The period prior to ascent was characterized by elevation of both CA in the blood of athletes in the 1st group, as compared to the physiological normal range. After the ascent, E and NE concentrations increased and exceeded significantly the baseline levels. Excretion of E, NE and dopa in urine and parameters of relative CA synthesis before the ascent were on the physiological level, but after descent from the peak there was increase in CA excretion in urine, particularly E and NE. Parameters of relative CA synthesis after the climb did not exceed the physiological norm.

Table 1. CA levels in blood ( $\mu\text{g}/\text{l}$ ) and urine ( $\mu\text{g}/24\text{ h}$ ) in 1st group of athletes ( $M \pm m$ )

Parameter	Before ascent		After ascent	
	blood	urine	blood	urine
E	$0.92 \pm 0.01$	$10.5 \pm 1.35$	$1.25 \pm 0.04$	$15.0 \pm 1.19$
NE	$1.86 \pm 0.07$	$26.9 \pm 1.20$	$2.18 \pm 0.11$	$34.3 \pm 2.06$
DA	-	$244.0 \pm 8.60$	-	$261.3 \pm 7.40$
Dopa	-	$26.0 \pm 1.80$	-	$28.6 \pm 2.21$
E/NE	$0.74 (100\%)$	$0.39 (100\%)$	$0.85 (115\%)$	$0.44 (112\%)$
E/NE	-	$0.11 (100\%)$	-	$0.13 (119\%)$
NE/DA	-	$0.48 (100\%)$	-	$0.14 (97\%)$
DA/dopa	-	-	-	-

Note: A dash signifies that the parameter was not determined by given method.

\* $P < 0.02$

\*\* $P < 0.05$

Thus, SAS activity did not exceed the normal physiological range in the 1st group of athletes during the training rally at high altitude. The high E and NE levels in blood before the ascent were probably related to presence of altitude hypoxia [11, 13], as well as the emotional state of participants preparing for the climb. Release of CA into blood and intensification of their excretion after the climb, which occurred without intensification of relative synthetic activity (probably due to discharge from the pool) probably due to the combined

effect of hypoxia and the physical load during the ascent, were indicative of high degree of conditioning and high adaptability of the SAS of these athletes.

Table 2. Excretion of CA and dopa in urine ( $\mu\text{g}/24 \text{ h}$ ) in 2d group of athletes ( $\text{M}\pm\text{m}$ )

Parameter	Before passage		After passage	
	men	women	men	women
E	$20.19 \pm 1.26$	$16.9 \pm 1.56$	$20.58 \pm 0.86$	$22.08 \pm 1.61^{**}$
NE	$29.6 \pm 1.10$	$32.5 \pm 3.87$	$47.6 \pm 1.97^*$	$47.6 \pm 1.09^{**}$
DA	$172.95 \pm 6.12$	$155.0 \pm 9.65$	$142.72 \pm 6.06$	$149.8 \pm 6.3$
Dopa	$49.5 \pm 1.51$	$47.6 \pm 4.96$	$33.92 \pm 2.36$	$57.2 \pm 4.10$
E/NE	$0.68 (100\%)$	$0.52 (100\%)$	$0.43 (64\%)$	$0.46 (89\%)$
NE/DA	$0.17 (100\%)$	$0.21 (100\%)$	$0.33 (194\%)$	$0.32 (151\%)$
DA/dopa	$3.49 (100\%)$	$3.40 (100\%)$	$4.23 (121\%)$	$2.60 (77\%)$

\*  $P < 0.01$

\*\*  $P < 0.05$

Examination of the 2d group of athletes before the passage revealed elevated excretion of E in urine (men), whereas excretion of other CA and dopa was in the range of the physiological norm. The high level of E was apparently attributable to the same causes as in the 1st group of athletes. After the passage, there was increase in NE excretion, E excretion remained high in the presence of somewhat low excretion of DA and dopa in the men. The decline of E/NE ratio, in comparison to the baseline, was indicative of prevalence of activity of the mediator element of the system. This was also confirmed by the increase in relative NE (NE/DA) and DA (DA/dopa) synthesis.

After the ascent, women presented an increase in excretion of E, NE and dopa in the presence of also prevalent (though to a lesser extent) activity of the transmitter element of the SAS, and the increased relative activity of NE synthesis (NE/DA) was associated with decline in relative activity of DA.

Thus, for the 2d group of athletes, exposure to high altitude was associated with activation of the transmitter element of SAS and intensification of CA synthesis, which was also due to the combined effect of hypoxia and physical loads. The differences in this system's reactions between men and women is probably related to differences in loads and individual reactivity of the SAS in men and women.

It is known that the course and outcome of hypoxia are determined by the degree of oxygen deficiency, on the one hand, and base reactivity of the body, on the other. Under hypoxic conditions there is functional change in different systems of the body, as a result of which several adaptive and compensatory reactions are triggered, which are directed toward elimination of hypoxia, tolerance to which depends on the state of the body's regulatory systems [4, 5]. Expressly the SAS, its transmitter NE and E secreted under the influence of its impulses, as well as adrenergic elements of the reticular formation, activate the cerebral cortex, enhance reactivity of all its analyzers, mobilize energy resources (carbohydrates and lipids) and their utilization. They stimulate activity of the cardiovascular and respiratory systems, improve muscular

efficiency and trigger the second adaptation system, the pituitary-adrenal cortex system, which enhances the effects of the sympathetic nervous system and maintains its activity by secreting glucocorticoids [6]. Thus, changes occur that adapt the body to new conditions (in particular, hypoxia) and help retain homeostasis [3].

The combination of hypoxia and physical loads inherent in the work of mountain climbers causes intensification of SAS activity, and it is known that physical loads activate the SAS under ordinary conditions [2, 8, 9], thereby improving adaptability of a hypoxia-conditioned organism and, at the same time, increases the oxygen deficiency and its uptake by tissues, which is a negative feature to some extent. Our findings revealed the extent to which the level of SAS activation and metabolic processes in the system differ. Thus, on the one hand, adaptation to hypoxia enhances resistance to loads, causes less discharge of transmitter (with a load) and probably faster termination of its effect [7]; on the other hand, it intensifies not only discharge, but synthesis. Thus, the combination of motor activity and high-altitude should be metered, while exposure to a set of specific physical loads must be included in the overall training for development of adaptation reactions to hypoxia, as well as investigation of SAS activity as a diagnostic test of conditioning of participants of expeditions in difficult climate conditions.

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DETERMINATION OF INCREMENT OF BACILLUS SUBTILIS BIOMASS IN WEIGHTLESSNESS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 24 Nov 83) pp 63-65

[Article by F. Bergter, D. Harz, P. J. Muller, K. Mund, U. Gunther, T. Hesse, R. Hartmann, G. Wanke, M. G. Tairbekov, G. P. Parfenov and A. I. Pakhomov (GDR, USSR)]

[English abstract from source] This paper presents the results of a microbiological experiment carried out by the Soviet and GDR scientists onboard Salyut-6. The experiment was performed using a *Bacillus subtilis* suspension in the Jena unit. The purpose of the experiment was to study the time-course variations of the cell biomass increase in zero-g. The cell culture development was measured with respect to the utilization rate of glucose or casein hydrolysate in the nutrient medium and the rate of protein accumulation in cells. It has been shown that the rate of biomass increment in zero-g lags behind the 1 g level. It can be concluded that the decreased metabolic activity of bacterial cells in zero-g is associated with changes in the cell population distribution and physicochemical parameters of the nutrient medium.

[Text] The coefficient of biomass increment is a variable determined by the equation  $y = \Delta X / \Delta S$ , where X is total yield of biomass and S is the amount of substrate used.

The increment coefficient is an integral parameter indicative of the degree of efficiency of metabolic processes in cells per unit nutrient substrate used. This parameter is the result of all pathways of energy and mass metabolism occurring in the cell, and it permits quantitative evaluation of the rate of growth processes in the cell under different ambient conditions. A comparative study of the intensity of bacterial cell growth and reproduction in weightlessness and on earth was the main purpose of the "Bacterial Metabolism" experiment performed jointly by USSR and GDR specialists. This study was pursued on the Salyut-6 orbital station during a visit to it by an international crew, with the participation of a GDR cosmonaut.

#### Methods

Concurrently with the main study conducted in weightlessness, there were two ground-based control experiments: at the Baykonur cosmodrome (USSR) and in

the laboratory of the Institute of Microbiology and Experimental Therapy in Jena (GDR).

The onboard Jena instrument was used in both the main and control studies; it was developed at the Research Center of Molecular Biology and Medicine (GDR). The instrument consists of 5 self-contained sealed chambers (each 4.5 cm<sup>3</sup> in size), in both ends of which sealed glass 0.5 cm<sup>3</sup>-ampules were installed. One of the ampules was filled with spores of a culture of *Bacillus subtilis* IMET 119 totaling  $2 \cdot 10^7$  in physiological NaCl solution and the other contained a fixing agent, 1% formaldehyde. There were 2 metal screws on the surface of the chambers over each ampule: a light one to break the ones with bacterial culture and a dark one, to break the ampules with fixing agent.

In the base state, each of the cylindrical chambers contained 1.5 ml glucose and casein hydrolysate (the concentrations are listed in the Table).

Glucose and casein hydrolysate content in chambers of Jena instrument

Chamber No	Glucose, g/l	Casein hydrolysate, g/l
1	0	0
2	1.25	1.25
3	2.50	2.50
4	3.75	3.75
5	5.0	5.0

Mineral salt content in the solution was so estimated as to have the following salt concentrations (in g/l) established after mixing the bacterial suspension with nutrient solution at the start of the test:  $\text{KH}_2\text{PO}_4$ --2.72,  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ --7.16,  $\text{NaCl}$ --5.0,  $\text{Na}_2\text{SO}_4$ --1.07,  $\text{NH}_4\text{Cl}$ --0.535,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ --0.406,  $\text{FeCl}$ --0.0054,  $\text{MgCl}_2$ --0.0039 at pH 6.8.

After opening the vials with spore suspension, each chamber should have contained 1.75 cm<sup>3</sup> air. On the basis of the results of preliminary studies, the concentrations of glucose and casein hydrolysate were

taken in a proportion with which we expected reduction of nutrient substrate to be a linear function of biomass yield.

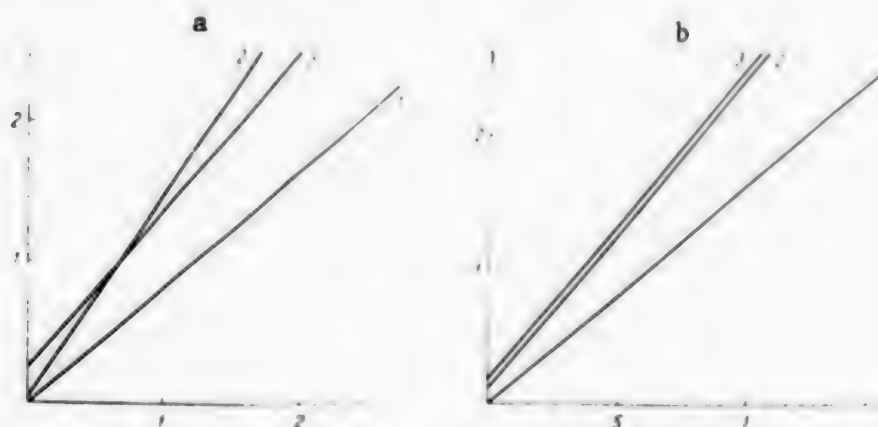
All of the procedures to prepare for the experiment, fill and sterilize the chambers were performed at the Institute of Microbiology and Experimental Therapy in Jena, GDR.

All 3 instruments were filled and placed in a transportation container 9 days prior to lift-off and stored during transportation at +4°C. Two instruments were delivered to the launching pad 2 days prior to lift-off. On the same day, one of the them was placed aboard the cargo craft and the other left on the ground for the control experiment. The experiment in weightlessness was begun a few hours after transferring the instrument to Salyut-6. Release of bacterial spores into the culture medium was considered the start of the experiment. The procedure was performed by the GDR cosmonaut, and for this purpose he had to turn the light screw clockwise to the stop in all five cylinders. The instrument was then agitated 5 times vertically; the shaking procedure was repeated every 12 h. The experiment lasted 62 h. It ended by fixing the contents of all chambers, which was done by turning the dark screw clockwise to the stop. After fixing, the instrument was shaken 5 times vertically, the lid was closed and it was packed for transportation to earth.

Ground-based control experiments were performed using an identical protocol at a 5-h lag, which was needed to receive information from the orbital station about the next operation. At the landing site, the experimental and control instruments were put in a transportation container (at a temperature of +4°C) and shipped to GDR.

## Results and Discussion

Postflight analysis of the suspension of bacterial cells in all three instruments was performed in a laboratory of the Institute of Microbiology and Experimental Therapy in Jena. The following cell characteristics were determined: density of suspension, protein content, concentration of glucose and total amino acid content. Density of the bacterial cell suspension was determined spectrophotometrically according to absorption coefficient at a wavelength of 470 nm. Protein content of cells was assayed by the method in [2]. For this purpose, 0.1  $\mu\text{l}$  suspension was applied to filter paper and stained with bromophenol blue. After elution, we measured the amount of dye absorbed by protein at a wavelength of 598 nm. Glucose concentration in the suspension was determined by the peroxidase-glucosidase method by means of the color reaction with participation of O-dianisidine. Fermognost reagent manufactured by the Dresden Chemical-Pharmaceutical Plant (GDR) was used as a test for sugar. Total amino acid content was measured by the method in [3] using ninhydrin.



Accumulation of protein in cells as a function of outlay of glucose (a) and casein hydrolysate (b). X-axis, concentration of glucose and casein hydrolysate; y-axis, concentration of protein ( $\text{g/l } 10^{-1}$ ). 1--flight, 2 and 3--control tests in Baykonur and Jena, respectively

The intensity of increment of bacterial biomass per unit nutrient substrate used--glucose or casein hydrolysate--was calculated using the formula given at the beginning of this article.

Since preliminary tests established that there was a linear function between these processes, the biomass increment for each chamber can be determined with the equation,  $Y = BX + A$ , where Y is mass of produced cells as determined by the amount of protein or optical density; X is substrate used, as determined

by decrease in glucose or casein hydrolysate; B is the coefficient of biomass increment; A is the point of intersection of the biomass increment curves and y-axis.

Biomass increment was calculated by measuring the density of the cell suspension and by the protein content of cells. The Figure illustrates plots of accumulation of protein in cells by *Bacillus subtilis* cultures as glucose and casein hydrolysate were utilized.

As can be seen from this figure, the intensity of biomass increment in weightlessness was lower than in the control tests, i.e., the rate of growth and reproduction of *Bacillus subtilis* cells was lower by about 30% in weightlessness than on the ground. The influence of weightlessness on distribution of cells in suspension could be the cause of this significant difference between the experiment and control. As we know, many populations of unicellular organisms develop specific types of distribution in liquid media, which are maintained for a long time by active gravity-dependent bioconvection [1, 4]. Since there is no convective movement at zero G, we could expect change in growth rate of such populations due to change in concentration and chemical gradients.

Thus, while it is rather clear that gravity has no effect on intracellular processes in free-living isolated organisms, growth and developmental distinctions of populations of unicellular organisms require further experimental investigation.

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## INVESTIGATION OF MICROFLORA OF CHUFA, A POTENTIAL HIGHER PLANT COMPONENT OF BIOLOGICAL LIFE-SUPPORT SYSTEMS FOR MAN

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 3 Feb 84) pp 65-68

[Article by L. S. Yunusova and N. A. Drugova]

[English abstract from source] The microbiological characteristics of the chufa plant grown alone or in combination with wheat and vegetables were investigated. The results showed that the bacterial flora of chufa plants did not change significantly. The bacterial count decreased during nodule formation. The number of actinomycetes increased when chufa and wheat plants were grown together. By the end of vegetation fungi were accumulated in the chufa rhizosphere. Among the physiological groups involved in the transformation of nitrogen-containing compounds ammonifiers were predominant. At the germination stage ammonification and denitrification processes increased, cellulose-decomposing bacteria appeared, oligonitrophilic forms occurred. From the microbiological point of view chufa plants can be used in the higher plant component of the biological life support system.

[Text] When developing biological life-support systems (BLSS) for man, it is necessary to take into consideration exchange of microflora between different elements of the system [3]. Since the higher autotroph element would include various plants, it is necessary to explore the possibility of joint cultivation of specific plant species in a greenhouse. In this regard, one of the important questions is to examine the distinctions of microbial components of all potential elements referable to higher plants as applied to BLSS for man. We previously studied the qualitative and quantitative composition of microflora of some vegetables and grain crops that could, because of their characteristics, be included in the higher plant element [4].

Our objective here was to investigate the dynamics of microorganism population size during substrate-free cultivation of chufa\* plants, their qualitative and quantitative composition.

\*Chufa [*Cyperus esculentus* L.--rushnut] is an oil-bearing plant of the Cyperaceae family, the edible portion of which are tubers formed on the plant roots. Its nutrient value is attributable to high fat and protein content.

## Methods

Chufa plants grown by a substrate-free method on Chesnokov solution, which was not changed, with 18-h and 24-h illumination in monoculture and with 24-h exposure to light together with wheat and an assortment of vegetables (carrots, beets, peas, cabbage and lentils) were the objects of microbiological investigation.

The plants were raised in experimental vegetation units (EVU) under artificial light from KG-1000-5 lamps with  $110-220 \text{ W/m}^2$  PAR [photosynthetically active radiation] intensity of radiation flux on the level of the planted area. Infrared irradiation did not exceed 60% of PAR irradiation. Root systems were automatically soaked with nutrient solution every  $16 \pm 1$  min. Solution temperature was  $19 \pm 1^\circ\text{C}$ . In the course of the studies, we constantly adjusted mineral nutrient content of solutions. Just prior to planting in the EVU, the seeds were treated with tetramethyluram disulfide (TMD)

A conventional method [2] was used to examine concomitant microflora. Samples of nutrient solutions and plant roots served as base material for these studies. Washings from roots were recovered by vigorous mixing of plant material in sterile water (1 g roots/100 ml water) for 15 min on an electromagnetic mixer. For better desorption of microorganisms from the surface, chufa roots were cut up with scissors under sterile conditions. The amounts to be used for inoculation and cultivation were determined experimentally. Three to five replicas were used for microbiological inoculations. The Koch dish method on elective nutrient media was used for quantitative assay of bacteria--on beef-extract and cabbage agar (BEA and CA), actinomycetes--on starch-ammonia agar (SAA), mold fungi and yeast-like organisms--on acidulated Czapek medium. Unconcentrated Ashby medium was used for demonstration of oligonitrophil forms of microorganisms. Maximum dilutions in liquid nutrient media were used for group analysis of the following physiological groups of microorganisms: ammonifiers--on peptone water (PW), denitrifying bacteria--on Gilbey medium, aerobic cellulose-digesting bacteria--on Voznyakovskaya's medium.

Reliability of results was determined by the statistical methods used in microbiological investigations.

## Results and Discussion

Chufa microflora was studied in the different phenophases: I--sprouting stage (7 days), II--shoots, III--start of mass-scale tuber formation, IV--industrial ripeness.

According to our results (Tables 1 and 2), the conditions of growing chufa plants (in monoculture and jointly with wheat or different vegetables on the same nutrient solution) did not alter appreciably the nature of development of bacterial flora. The number of bacteria decreased in the period of intensive tuber formation, and this is apparently related to minimum release by plants of nutrients into the medium. The dynamics of actinomycetes development were similar to bacterial development only in the chufa monoculture. Joint cultivation of chufa and wheat led to increase in number of actinomycetes, particularly on chufa plant roots. This was probably due to the influence of the microbial cenosis of wheat. We previously had observed active development of actinomycetes

when cultivating wheat [1]. In all experimental variants, there was accumulation of fungi in the chufa rhizosphere toward the end of vegetation, particularly when cultivation of chufa was combined with wheat and vegetables.

Table 1. Dynamics of development of microflora concomitant with chufa plants (millions per gram roots)

Chufa pherophase	Bacteria on BEA	Bacteria on CA	Fungi	Actinomycetes	Oligonitrophils	Cellulose-digesting bacteria
Chufa monoculture with photoperiod						
I	47,85 ± 2,19	131,25 ± 11,46	ND	ND	8,70 ± 0,93	
II	181,75 ± 13,48	204,00 ± 14,97	0,010 ± 0,001	1,250 ± 0,11	16,48 ± 1,28	0,450
III	1,07 ± 0,10	14,80 ± 1,22	0,010 ± 0,001	0,250 ± 0,05	50,07 ± 2,58	0,025
IV	14,78 ± 1,22	171,75 ± 13,14	0,041 ± 0,002	2,670 ± 0,19	131,00 ± 13,2	0,060
Chufa monoculture with 24-h illumination						
I	47,85 ± 2,19	131,25 ± 11,45	ND	ND	8,70 ± 0,93	
II	12,20 ± 1,10	12,53 ± 1,06	0,012 ± 0,001	1,067 ± 0,119	81,50 ± 9,02	0,0003
III	4,20 ± 0,20	6,21 ± 0,25	0,050 ± 0,002	9,667 ± 0,114	100,75 ± 10,0	0,0400
IV	11,88 ± 1,09	33,38 ± 1,82	0,157 ± 0,012	1,000 ± 0,120	12,85 ± 1,13	2,5000
Chufa + wheat						
I	45,18 ± 2,13	57,30 ± 2,76	ND	ND	36,73 ± 1,92	
II	16,73 ± 1,29	17,60 ± 1,32	0,006 ± 0,0009	2,75 ± 0,17	18,70 ± 0,60	0,600
III	4,82 ± 0,22	13,68 ± 1,17	0,136 ± 0,009	4,26 ± 0,20	11,70 ± 1,08	0,045
IV	20,40 ± 1,43	116,50 ± 1,07	0,295 ± 0,019	6,38 ± 0,29	93,00 ± 11,1	0,060
Chufa + assortment of vegetables						
I	45,18 ± 2,12	85,75 ± 9,32	ND	ND	36,72 ± 1,92	
II	27,40 ± 1,66	19,08 ± 1,38	0,037 ± 0,001	2,250 ± 0,15	67,25 ± 8,20	0,450
III	17,78 ± 1,32	39,00 ± 1,97	0,151 ± 0,008	1,625 ± 0,130	11,05 ± 1,05	0,003
IV	92,25 ± 9,60	203,00 ± 14,3	0,247 ± 0,018	ND	113,25 ± 10,6	0,015

Key for this and Table 2:

-) no cellulose-digesting bacteria demonstrated

Key: ND) not demonstrated

ND) none demonstrated

Of the examined physiological groups of microorganisms involved in conversion of nitrogen-containing compounds, there was prevalence in the chufa plants, as in all higher plants examined, of the ammonification group, i.e., bacteria capable of utilizing, as a source of nitrogen, protein and other organic nitrogen compounds (Figure 1). Processes of ammonification and denitrification (reduction of nitrates to molecular nitrogen) are intensified at the shoot phase, after which the number of microorganisms involved in these processes becomes stabilized. The findings are somewhat different in nutrient solutions due to regular correction of solutions by addition of mineral nutrients as they are absorbed by plants (Figure 2). In both plant roots and nutrient solutions, in all variants, we demonstrated oligonitrophils, i.e., bacteria capable of growing with infinitesimal amounts of nitrogen. This group of microorganisms can include sporogenic and nonsporogenic bacteria and mycobacteria. It is known that oligonitrophils can also fix atmospheric nitrogen. Thus, investigation of the dynamics of population of microorganisms involved in nitrogen metabolism revealed energy processes of conversion of nitrogen-containing compounds.

Table 2. Dynamics of development of concomitant microflora in nutrient solution (thousands/ml solution)

Chufa pheno-phase	Bacteria on BEA	Bacteria on CA	Fungi	Actinomycetes	Oligonitrophils	Cellulose-digesting bacteria
Chufa monoculture with photoperiod						
I	26,88±1,64	4,83±0,22	0,060±0,008	1,05±0,10	3,80±0,19	—
II	586,75±24,22	684,00±26,15	0,017±0,001	7,50±0,86	268,75±16,39	0,045
III	4,40±0,21	47,75±2,22	0,034±0,006	0,020±0,001	254,00±15,94	0,009
IV	57,25±7,56	107,30±11,96	0,341±0,009	14,00±1,37	125,00±11,18	0,250
Chufa monoculture with 24-h illumination						
I	4840±220	3672,5±191,6	0,039±0,007	ND	2613±186,4	—
II	128,00±11,31	36,75±1,91	0,012±0,003	0,86±0,11	36,25±1,90	0,065
III	12,03±1,096	18,05±1,34	0,856±0,058	30,00±6,32	20,35±1,42	2,500
IV	17,65±1,330	31,90±1,79	0,488±0,018	9,30±2,23	13,25±1,15	0,250
Chufa + wheat						
I	27,33±1,56	12,40±1,11	0,042±0,006	ND	12,10±1,10	—
II	30,08±1,73	41,00±2,31	0,120±0,013	6,50±0,80	13,53±1,16	0,600
III	370,00±19,24	238,00±15,11	0,228±0,017	41,25±2,03	146,75±12,11	0,075
IV	25,40±1,59	30,23±1,74	0,267±0,190	39,67±2,30	145,00±13,90	25,000
Chufa + assortment of vegetables						
I	27,23±1,65	12,40±1,11	0,042±0,006	ND	12,10±1,10	—
II	21,15±1,45	16,85±1,30	0,092±0,011	5,00±0,70	17,05±1,17	0,750
III	12,18±1,10	27,85±1,67	0,203±0,016	130,00±11,4	9,03±0,95	0,096
IV	30,20±1,74	30,25±1,74	0,071±0,008	26,50±1,62	8,23±0,92	0,026

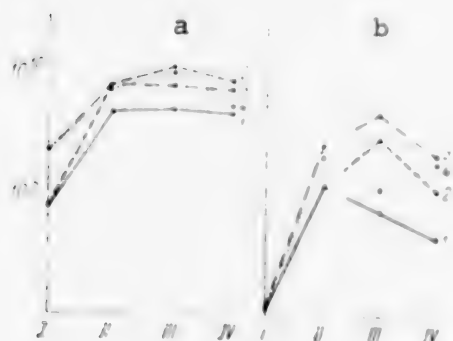


Figure 1.

Dynamics of development of ammonification (a) and denitrification (b) bacteria on chufa roots; x-axis phase of chufa plant development; y-axis, number of microorganisms per gram roots

- 1-2) chufa monoculture with photoperiod and around the clock illumination, respectively
- 3-4) chufa together with wheat and with vegetables

of plant development, since the cellulose they contain becomes the source of nutrition for microorganisms that contain the hydrolytic enzyme, cellulase,

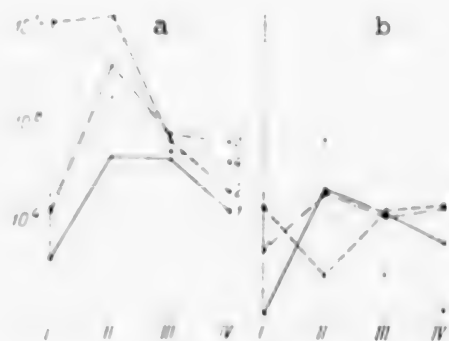


Figure 2.

Dynamics of development of ammonification and denitrification bacteria in nutrient solutions; y-axis, number of microorganisms per ml solution. Other designations are the same as in Figure 1

Representatives of the group of cellulose-digesting microorganisms usually appear at the late stages



after root cells die off. However, cellulose-destroying microorganisms were found on chufa roots already at the shoot stage, which is indicative of possible temporary disturbances of physiological processes in the plants. Thereafter, the number of microorganisms of this group became stabilized.

Thus, the findings indicate that, from the microbiological point of view, chufa plants can be included among the higher plants used in BLSS for man, in both monoculture and combined with grain and vegetable plants.

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INVESTIGATION OF DISTINCTIONS REFERABLE TO GROWTH, DEVELOPMENT AND METABOLISM OF CLOSTERIOPSIS ACICULARIS ALGAE WHEN CELLS ARE LIMITED IN NITROGEN AS RELATED TO BIOLOGICAL LIFE-SUPPORT SYSTEMS FOR MAN

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 30 Sep 83) pp 69-73

[Article by A. A. Antonyan, M. A. Levinskikh and N. I. Sukhova]

[English abstract from source] The growth, development and metabolism of a new form of green unicellular algae *Closteriopsis acicularis* were investigated from the point of view of their potential use in the biological life support system (BLSS). Their growth and biochemical characteristics were studied as a function of nitrogen supply. During the nitrogen-deficiency cultivation the growth rate remained unchanged, cell division and biomass increment was uncoupled, and carbohydrate formation was predominant. The content of carbohydrates increased at the expense of assimilable fractions, particularly starch. These data can be used in the selection of an optimum nitrogen level for the case of algal continuous cultivation, in the calculation of material flow charts, and in the development of diets that can be provided by a BLSS incorporating *Closteriopsis acicularis*.

[Text] Induction of specific changes in composition of algal biomass in the direction of augmenting the amount of nitrogen-free substances--lipids or carbohydrates--in it is one of the means of optimizing the features of the algal component of biological life-support systems (BLSS) for man [1, 2, 16, 18, 22]. Investigation of these matters is particularly important because the biomass of unicellular algae presently used in BLSS contains excessive amounts of protein, so that it cannot be used as part of the diet in large quantities or provide for a balance of the basic flow of substances in the system without exogenous corrections.

The feasibility of effecting specific changes in biosynthesis of algae by altering medium conditions, in particular, by limiting nitrogen in culture, has been investigated rather well in several strains of *Chlorella* sp-k [7, 10, 13, 17, 21, 22, 26, 27] and *Chlamydomonas reinhardtii* [4, 15], although there are some disagreements with respect to interpretation of dissociation of cell functions [5]. In particular, it was shown that the direction of metabolic

processes may vary--carbohydrate or lipid--when cells are limited in nitrogen, depending on the genetic distinctions of algae [11, 13].

It is of definite interest to investigate these properties, in comparison to *Chlorella* sp.-k, in one of the new forms of *Closteriopsis acicularis* algae of the *Ankistrodesmaceae* family, *Chloropyceae* class, which we introduced into intensive culture and, because of several physiological and ecological properties, has made a name for itself as a potential component BLSS for man. The choice of this form for use in biological systems is also attributable to the high carbohydrate content of its biomass [12, 23]. However, we found no information in the literature concerning variability of metabolism as a function of nitrogen nutritional conditions. Information pertaining to a close genus, *Ankistrodesmus*, indicates that lability of metabolism as a function of cultivation conditions is also inherent in them [20].

Our objective here was to investigate the direction of metabolism in *Closteriopsis acicularis* algae with limitation of nitrogen in cells, the possible range of its changes, link between these changes and rate of growth and reproduction of cells as applied to BLSS for man.

#### Methods

Algae were cultivated in accumulating and continuous modes with use of bubbling and rotating types of culture equipment. In all of the experiments, the basic ambient parameters were stabilized in the following ranges: suspension temperature 36-37°C, concentration of carbon dioxide 2-5%, oxygen 20-25%, intensity of illumination 200 W/m<sup>2</sup> PAR [photosynthetic activity of radiation], rate of stirring suspension 30-35 r/min. We used the following nutrient medium (g/l) in experiments to examine the effect of nitrogen deficiency on algal growth and metabolism: KNO<sub>3</sub>--2.9, KH<sub>2</sub>PO<sub>4</sub>--0.55, MgSO<sub>4</sub>·7H<sub>2</sub>O--0.47, Arnon solution of trace elements with iron--1 ml/l medium. All experiments in this series were performed in the accumulative mode with gradual (as growth progressed) yield of culture with nitrogen deficiency in medium. Growth rate was determined by the number of cells in a Goryayev chamber (million/ml) and culture yield, by the dry matter (g/l/h, day). Protein content of biomass was calculated from total nitrogen with use of a coefficient of 6.25 [9]. Lipids were extracted by the Folch method [25] and assayed by a weighing method; carbohydrates, according to reducing activity using anthrone reagent [6, 8]. Sugars were separated after Kizel' [19]. The data were submitted to statistical processing with use of Student's criterion and confidence interval with P = 0.95 [24].

#### Results and Discussion

At first, we examined growth rate and composition of *Closteriopsis* biomass with cultivation of algae in the presence of full amounts of all biogenous elements. These data served as the baseline for evaluation of extent of variability of the studied characteristics of the cultures with nitrogen deficiency.

As a result of the experiments, it was shown that when the full amount of nitrogen and other mineral nutrients (phosphorus, sulfur, magnesium) was present over a wide range of concentrations, the growth rate was equally high throughout the linear segment of the curve, constituting a mean of 0.405 g/l/h

(Figure 1a). Slower algal growth during accumulative cultivation was demonstrable, according to biomass increment, with algal suspension density of 14-16 g/l and it was apparently due to limiting the amount of light for the culture. Mean 24-h algal yield constituted  $9.7 \pm 0.67$  g/l, which was 8.6 times more than the yield of the same algae in extensive culture, and it was close to the productivity of some strains of *Chlorella* sp-k under analogous cultivation conditions [14]. The build-up of cells paralleled the increase in dry biomass until culture growth stopped.

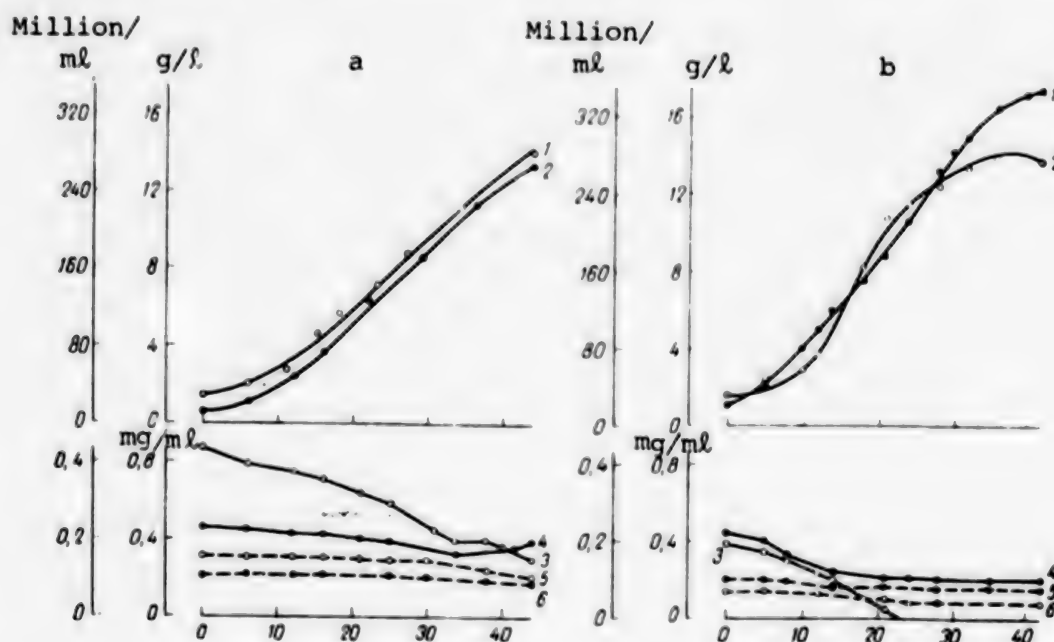


Figure 1. Dynamics of rate of growth in biomass (1), number of cells (2), concentrations of nitrogen (3), phosphorus (4), magnesium (5) and sulfur (6) in intensive accumulative culture with full supply of nitrogen (a) and with a nitrogen shortage (b) in the culture

X-axis, time of cultivating algae (h); y-axis, top: number and dry weight of algal cells; bottom: concentration of nitrogen, phosphorus, sulfur and magnesium in medium

Under these conditions, biomass nitrogen content was 55-62 mg/g dry matter, which corresponded to fluctuation of protein content of cells in the range of 36-39%, lipids from 13 to 16% and carbohydrates 33 to 38%, including starch 24 to 30% scaled to dry matter. The composition of *Closteriopsis* biomass was found to be similar to a form of algae, *Chlamydomonas reinhardtii*, that we had studied previously [2, 15]. Presence of all other elements in the medium indicates that cells were limited only in nitrogen in the course of accumulating cultivation of algae, which did not lead to decline of growth rate (Figure 1b). After depletion of nitrogen in the medium, cell growth continued due to redistribution of intracellular reserve and it held at a level of  $0.41 \pm 0.61$  g/l/h until cell nitrogen content dropped to 27 mg/g dry biomass. Further decline of nitrogen content of cells from 27 to 20 mg/g corresponded to the lag



phase, and with decline of nitrogen to 18-15 mg/g, to the period of arrested growth and passage of culture to a plateau. The rate of increase in number of cells in the linear segment of the growth curve remained unchanged for 4 h after depletion of nitrogen from the medium, constituting a mean of 8 million/ml/h. There was slowing of the process of cell division with decline of nitrogen content to 30-35 mg/g. Total arrest of cell division was recorded in all experiments 4-5 h sooner than arrest in build-up of biomass under these conditions, which is indicative of some dissociation of cell functions when they are deficient in nitrogen. This corresponded to 20-25 mg/g dry weight, and we used this as the minimal amount for normal growth and development of algae. It was considerably lower than determined for some of the other algal forms we had studied--*Chlorella chlamydomonas reinhardtii*, *Spirulina platensis* and *Euglena gracilis* [3].

Table 1. Range of fluctuations in biochemical composition of *Chlorella* sp-k and *Closteriopsis acicularis* on different segments of growth curve with nitrogen deficiency (% dry matter)

Algae	Linear segment		Lag phase		Arrested growth	
	protein	carbohvd.	protein	carbohvd.	protein	carbohvd.
<i>Chlorella</i> sp-k	55-32	16-48	32-25	48-50	25-20	50-52
<i>Closteriopsis acicularis</i>	38-18	32-55	18-15	55-60	15-12	60-65

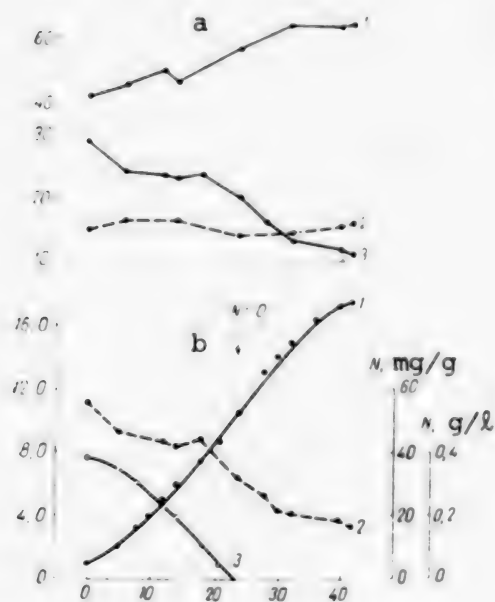


Figure 2.

Dynamics of composition of *Closteriopsis acicularis* biomass in accumulating mode of cultivation with shortage of nitrogen in medium

a) carbohydrate (1), lipid (2) and protein content of biomass during accumulating growth

The nature of changes in algal biomass in one of the experiments with limited nitrogen in cultures is illustrated in Figure 2, while the range of change in composition demonstrated at different segments of the growth curve in many experiments is listed in Table 1. These data indicate that metabolic changes occurred in algae mainly within the linear phase of growth due to decrease in protein content of cells, and they were directed toward increase in carbohydrate synthesis. Lipid content remained unchanged in the biomass. At the lag phase there was further decline of protein content with concurrent increase in total carbohydrates. When culture growth stopped (curve showing a plateau) the changes in biomass composition were insignificant and apparently due mainly to partial destruction of cell structures.

b) curve of algal growth (1), nitrogen content in cells (2) and medium (3)

The linear segment of the growth curve was of greatest interest for obtaining a high carbohydrate content in the biomass, since this was associated with high specific algal yield. For this reason, in the case of continuous and intensive culturing of *Closteriopsis* with nitrogen deficiency for use in BLSS, nitrogen supply for cells should be at least 20 mg/g. This permits maintaining carbohydrate biomass in the range of 50-55%, scaled to dry matter.

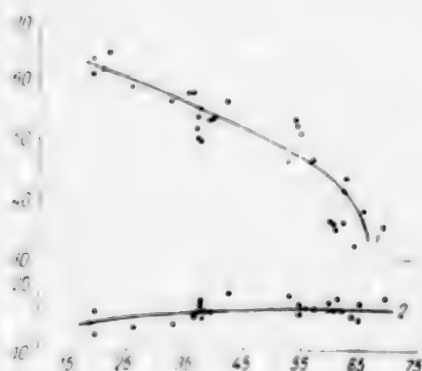


Figure 3.

Carbohydrate (1) and lipid (2) content as a function of biomass nitrogen content (according to data for all experiments)

X-axis, nitrogen content in algal biomass; y-axis, lipid and carbohydrate content (% dry matter)

Figure 3 sums up data for all experiments dealing with cultivation of *Closteriopsis* in intensive and extensive modes in the form of quantity of carbohydrates and lipids as a function of biomass nitrogen content. As nitrogen content of cells diminished from 70 to 15% of dry mass, we observed an increase in biomass carbohydrate content from 33-36 to 60-65% dry cell weight, i.e., by 2 times, which was associated with an equivalent decline of cell protein content. According to the data in Figure 3, lipid content held at virtually an unchanged level--15-17%.

Concurrently with increase in total carbohydrate content, there were changes in some of their fractions (Table 2)

The changes in different carbohydrate fractions in the course of accumulating growth of algae were quite different in relation to total carbohydrate content. For example, at the end of the linear segment of the growth curve starch content constituted more than 87% of total carbohydrates, versus 65% with normal nitrogen content. The hemicellulose fraction, which normally constitutes 4.7% of the total, dropped to 2.5% with limited nitrogen supply to cells. Low-molecular polysaccharide content remained in the range of 6-7% of the total under these conditions, and it was unrelated to cell nitrogen content. The increase in carbohydrate content of biomass occurred mainly due to the starch fraction and, in part, monosaccharides and low-molecular polysaccharides, i.e., the arbitrarily "assimilable" carbohydrates. They totaled 44.8% of dry matter by the end of the phase of linear growth of algae, versus 28.2% under normal conditions.

Table 2. Amounts of main carbohydrate fractions at two phases of growth of *Closteriopsis acicularis* with nitrogen-deficient cells (results of 4 experiments; % dry weight)

Growth phase	Parameter	Monosaccharide	Low-molecular polysaccharides	Starch	Hemicellulose	Total carbohydrates
Linear	$\bar{x}$	2.2	2.4	40.2	1.10	47.4
	$\pm S$	0.20	0.25	4.6	0.08	4.2
Lag phase	$\bar{x}$	3.0	3.4	48.8	1.7	56.9
	$\pm S$	0.50	0.44	2.6	0.21	6.0

Thus, these studies with cultivation of *Closteriopsis* in an accumulating mode with a nitrogen deficiency revealed absence of decline of growth rate, presence of dissociation between cell division and accumulation of biomass, change in direction of metabolic processes in cells toward production of carbohydrates. It is important that the increase in carbohydrate synthesis occurred at the expense of assimilable fractions, mainly starch, which is important to evaluation of suitability of algal biomass for feed and food purposes, on the one hand, and BLSS for man, on the other. The nature of changes in growth characteristics and direction of metabolic processes in *Closteriopsis* cells as a function of conditions of limiting them in nitrogen is similar, as compared to *Chlorella* and *Chlamydomonas* algae, although *Chlorella* sp-k and *Chlamydomonas platensis* present some differences with respect to change in the various fractions of carbohydrates (for example, starch) when there is a shortage of nitrogen. The obtained data can serve as the basis for selection of an optimum mode of limiting nitrogen for cells in the course of continuous intensive cultivation of algae and in relevant calculations of material balance of *Closteriopsis*, as well as for planning diets in BLSS for man that include algae.

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FORMATION OF VOLATILE SUBSTANCES DURING POLYMER DESTRUCTION BY PSEUDOMONAS AERUGINOSA

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 15 Mar 84) pp 74-76

[Article by N. D. Novikova and S. N. Zaloguyev†]

[English abstract from source] The effect of biodestruction of paint-and-varnish coatings on the formation and evolution of toxic substances was studied. It was found that the multiplication of *Pseudomonas aeruginosa* on epoxide resins (EP-255+ EP-525/AK-070) modified the evolution of gases: that of acetone and n-butanone increased and evolution of xylene and ethyl benzene isomers decreased.

[Text] In recent years, there has been considerable increase in attention given to destruction of polymers under the effect of microorganisms. Many publications [1-3] have reported the possibility of change in physical and mechanical properties of materials and appearance of malfunction in diverse equipment as a result of reproduction of bacteria and fungi. It is rather important that, when microorganisms reproduce on materials, there can be a change in the latter and, as a result, intensive discharge of trace impurities into the environment. It is quite possible for new chemical impurities, which are not inherent in unadulterated materials, to appear in the emitted gases, and they may have an adverse effect on man.

There has been little study of the formation and emission of volatile toxic agents in the course of biodestruction of polymers. It has been shown, for example, that with use of cables and wires in regions with a tropical climate there is destruction of insulation, as a result of which hydrogen chloride is formed [2]. Biodestruction of phthalate plastics under the effect of *Pseudomonas aeruginosa* could lead to splitting of the alcohol radical and release of alcohols into the environment [5]. Many naturally occurring polymers (skin, silk, wool, etc.) are very susceptible to biodestruction, with formation of amino acids which release ammonia and volatile organic acids upon deamination [8].

It is particularly important to solve these problems for objects in an artificial habitat, in particular, spacecraft cabins. Appearance of changes in intensity and nature of gas emission from polymers as a result of reproduction

of microorganisms on them could worsen appreciably the living conditions for people. If condensation moisture, which could be instrumental in reproduction of microorganisms on polymers as was shown in [4], falls on the materials it could have a substantial effect on this process.

The difficulty of solving this problem is attributable to the fact that we do not presently have scientifically validated methodological procedures for studying volatile products formed when microorganisms multiply on materials. Nor has the composition of chemicals released by polymers been definitively identified.

Our objective here was to examine the composition of gas emissions from the polymers used the most widely in routine practice, in particular, a system of epoxy enamels EP-255 and EP-525 on AK-070 primer, bearing in mind the possibility of development on it of microorganisms of the species *Pseudomonas aeruginosa* (blue-green bacillus).

#### Methods

We tested samples of materials taken on the basis of  $1 \text{ m}^2/\text{m}^3$  volume of hypothetical room. The samples were placed in a vacuum desiccator equipped with a sample-collecting device of the membrane type. We poured a condensate of atmospheric moisture recovered in a study involving people in pressurized rooms on the bottom of the desiccator where the tested material was. We first added to the condensate a suspension of test microorganisms prepared so as to obtain an end concentration in the condensate of 20,000 bacterial cells per milliliter. The choice of condensate as medium into which a sample of tested material was placed was made due to the fact that when people are in sealed compartments where life-support systems operate, it is possible for condensation moisture to fall on interior surfaces. We prepared test samples as follows, in order to identify microimpurities in the experimental sample as opposed to those in unadulterate condensate and condensate infected with the test microorganisms, as well as microimpurities in the material: 1) the material was placed in a condensate of atmospheric moisture without the test microorganism; 2) an analogous sample of material was moistened with distilled water; 3) the condensate was infected with a suspension of test microorganism in the same concentration.

Samples prepared in this manner were kept at a temperature of  $20 \pm 2^\circ\text{C}$  for 22 days and at  $37 \pm 0.1^\circ\text{C}$  for 49 days. Gas samples were collected from the desiccators with medical 10-ml syringes and examined by the method of gas adsorption and gas-liquid chromatography [6]. The method of equilibrated vapor phase was used to examine the condensate [9].

Concurrently with testing of gas samples by the conventional methods of sanitation bacteriology, we counted the total number of test microorganisms of the species *Pseudomonas aeruginosa*.

#### Results and Discussion

It was determined that bacteria of the species *Pseudomonas aeruginosa* can reproduce in the presence of the epoxy enamel system. They multiplied more

intensively on this material at a temperature of 37°C. In the absence of the material, we also observed growth of *Pseudomonas aeruginosa* bacteria, which was more marked at 37°C, but toward the end of the experiment the bacterial count was higher with statistical reliability ( $P < 0.01$ ) in the experiment than in the control.

Concentration of volatile agents ( $\text{mg}/\text{m}^3$ ) when samples were kept at  $37 \pm 0.1$  and  $20 \pm 2^\circ\text{C}$

Composition of initial samples	Volatile compounds	Days kept at					
		$37 \pm 0.1^\circ\text{C}$			$20 \pm 2^\circ\text{C}$		
		7	21	49	7	14	22
Experiment: system of paint and varnish + condensate + bacteria	Carbon dioxide	nd	0.056	0.084	nd	nd	nd
	Acetone	14.80	25.00	73.10	10.2	27.2	93.5
	Toluene	4.52	4.80	24.00	2.40	2.40	2.88
	n-Butanol	1.60	3.36	6.90	—	1.84	1.61
	n- and p-Xylenes	19.80	11.44	41.60	0.52	2.08	3.38
	O-Xylene	4.60	2.97	11.60	0.99	—	0.99
	Ethylbenzene	13.60	7.48	27.20	1.02	1.40	2.72
Control: system of paint and varnish + distilled water	Carbon dioxide	nd	0.042	0.053	nd	nd	nd
	Acetone	17.00	8.20	3.91	6.1	18.9	79.9
	Toluene	11.50	15.50	26.40	1.9	1.44	4.32
	n-Butanol	2.07	1.80	1.84	nd	nd	1.38
	m- and p-Xylenes	23.14	27.00	61.10	0.52	2.60	8.06
	o-Xylene	5.61	9.90	26.40	0.99	0.99	1.98
	Ethylbenzene	17.00	39.00	42.50	1.02	2.04	5.78
Condensate	Ethanol	5.26	8.56	2.59	—	—	—
	Acetone	1.36	2.04	1.02	—	—	—
Condensate + bacteria	Carbon dioxide	nd	0.063	0.077	—	—	—
	Ethanol	4.61	9.20	8.55	3.95	3.50	5.95
	Acetone	1.19	2.56	1.26	1.70	1.40	2.31
	Carbon dioxide	nd	0.26	0.096	nd	nd	nd

Notes: nd--none demonstrated. Carbon dioxide concentration is given as a percentage.

The studies of kinetics of gas emission in a laboratory experiment were conducted by V. D. Yablochkin, and the results are listed in the Table. At temperatures of both  $18-20^\circ\text{C}$  and  $37^\circ\text{C}$ , the tested paint and varnish system emitted a complex combination of volatile compounds, which included carbon dioxide, acetone, toluene, n-butanol, isomers of xylene and ethylbenzene. However, neither the monomer epichlorohydrin nor components of the enamel hardener were demonstrable at either temperature. Thus, the set of products of gas emission from the paint and varnish system included organic solvents and carbon dioxide. Unadulterated condensate contained small amounts of ethanol and acetone.

Examination of the kinetics of gas emission from the paint and varnish system samples revealed that the concentrations of most volatile compounds increased with rise in temperature and increase in exposure time. Thus, a test of the atmosphere of the desiccator at  $20 \pm 2^\circ\text{C}$  revealed that acetone concentration increased to  $93.5 \text{ mg}/\text{m}^3$ , whereas in the control, where the sample of material was placed in distilled water, it was 1/20th of this value. Perhaps acetone



which is a component of enamel solvent, was formed because of biodestruction of the epoxy cover.

Examination of the kinetics of emission of n-butanol also revealed differences in rate of rise of its concentration, as compared to the control, which was more marked at 37°C. At this temperature, the concentration of n-butanol on the 49th day was almost 4 times higher than in the control, whereas at room temperature this parameter was only 20% above control values.

A study of the kinetics of emission of xylene and ethylbenzene isomers enabled us to detect differences in rate of rise in their concentrations between the experiment and control. On the 7th day, their concentrations in sealed compartments were the same, but already on the 13th day there was more intensive increase in the control, and on the 22d day the difference was 1.5-2 times greater. It may be that the effect of metabolic products of *Pseudomonas aeruginosa* on the surface of the enamel was one of the causes of slower release of volatile compounds in the experiment. We cannot rule out the possibility that there are compounds among the products of vital function of test bacteria that prevent free desorption of low-molecular volatile compounds from epoxy enamel. Indeed, analysis of the unadulterated condensate, as well as condensate with a sample of the enamel at 37°C revealed that formaldehyde concentration rose from 0.95 to 35 mg/l. It has been shown [7] that formaldehyde blocks desorption passages and, consequently, inhibits desorption of volatile compounds from polymers.

The effect on the surface of epoxy enamels of amines, which are metabolic products of microorganisms [10] may be another possible cause of decreased emission of volatile gases from materials, with consequent formation of a reticulate structure. Amines, in their usual role of hardening agents, interact with epoxy resins causing their additional "splicing" and lowering permeability of the polymer to gas. Indeed, analysis of the condensate revealed that, at a temperature of 20°C, total concentration of amines and ammonia dropped on the 22d day from 48.5 mg/l to traces in the experiment. At the same time, the levels of these compounds decreased only to 1/5th in the control, apparently due to adsorption by the desiccator walls.

Thus, it was established that when *Pseudomonas aeruginosa* bacteria reproduce on a paint and varnish system (EP-255 + EP-525 on AK-070 primer), there is change in intensity of gas emission into the environment. More emission of acetone and n-butanol, and less emission of xylene and ethylbenzene isomers was observed.

The results of our studies revealed that further in-depth and systematic investigation of nonmetal materials of different types is necessary, with use of a standard set of test cultures.

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## ONE ASPECT OF CREW TRAINING

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 12 Jan 84) pp 77-80

[Article by Ye. D. Kononov]

[English abstract from source] This paper describes the psychophysical training of crewmembers of flying vehicles who may find it necessary to cross a water boundary or to remain for long in water after emergency splash-down. In order to prevent attacks of fear and panic caused by such frightful things as whirlpools and convulsions, the paper presents data on the nature of turbulent processes in running water and the techniques of overcoming them as well as on the origin of convulsions in swimmers and methods of their alleviation. The paper also describes an easy method of how to have a rest when swimming that makes it possible to eliminate the feeling of fatigue by remaining motionless for an infinitely long time.

[Text] It is known that situational training of flight personnel includes, in particular, development of survival skills under extreme conditions after a splashdown or landing in inaccessible mountain, desert or tayga regions. The program was prepared on the basis of the recommendations of such specialists as P. Nesbit [2] and V. G. Volovich [1], whose developments were used in outfitting portable emergency supply kits (NAZ) and served as the basis for writing up standard self-rescue procedures for the crew. However, the consequences of an accident could compel members of the crew to overcome, either as a group or one by one, water barriers while swimming or to survive on water for a long time and, depending on the geographic latitude, season and other circumstances, it may be necessary to remain afloat without any special floating gear. And, since much importance is attributed to comprehensive training of crews, we considered it expedient to augment it with some elements of a psychophysiological program.

There is every reason to expect that such training is important to spacecraft crews.

A sociological and psychological study pursued on a large group of swimmers (interviews, questionnaires, analysis of written reports on why people, who knew how to swim, were found in a critical state, referable to more than 1000

cases) revealed that psychogenic stress and fear reactions were inherent in the absolute majority of those who had been swimming in natural bodies of water. At times these signs were so marked that they could be associated with a full-fledged clinical picture of emotional stress, ranging from signs of stupor or uncontrollable motor excitement to an attack of weakness and vomiting. As we know, such mental and autonomic reactions are concomitant with emotional stress whatever its origin, and they have been described extensively in the literature. But, while an individual under stress on land is seldom in danger for his life, the inability for mental self-control in the water can apparently end much more often with a tragedy.

It was learned that fear is formed under the influence of experience, impressions and knowledge gained from all sources of information. It may be overt and constant, or (in good swimmers) be localized in the subconscious and therefore the individuals may not be aware of it. By virtue of various causes, some people never do learn how to swim, acquiring anxiety because of stories about people who have drowned. Others, having learned to swim, have gained a shaky psychological victory over themselves: we found that even good swimmers capable of swimming for many kilometers and many hours may be unable to withstand the sudden thought: "What if I drown!", although they would deny any conscious fear in the water prior to the described incident. In such cases, the distance to shore was not necessarily measurable in kilometers or hundreds of meters, rather it was sometimes only tens of meters.

The admission by people that were highly ranked in sports swimming that when they swam into unfamiliar places, under adverse weather conditions and in certain other circumstances also sometimes experienced fear attacks was an unexpected disclosure. Having no reason to reject an anxious thought, the swimmers became captives of their own negative emotions and were on the brink of death.

The sensations of people who swim with less confidence are manifested by the fear of cramps, eddies and doubt in their own strength, and for this reason their psychogenic stress changes readily to panic at the slightest attendant factor.

Such lack of self-confidence is attributable to the fact that people do not acquire a modicum of applied information about physiological, physical and psychological phenomena as related to swimming. For this reason, the wrong and therefore harmful information becomes fixed in their consciousness to the effect that all rivers and bodies of water have a profusion of whirlpools and eddies that tow people under; that the cramps that swimmers develop in one limb could involve all muscles and "reach the heart," etc. All this develops a set for anxious expectation during swimming of imaginary danger and complications, and elicits stress.

Thus, it becomes apparent that, in order to prevent fear while swimming, one should first of all provide objective information about the phenomena that appear to be mysterious, foreboding and generate anxiety. Since the group to which this study is addressed apparently differs little in its "water" knowledge and skills from the general population, this article is informative and instructive in nature, since the success of overcoming a



crisis situation while swimming depends largely not only on practical training but on being informed.

What should one know about whirlpools? In stagnant water (lakes, ponds, reservoirs) where there is essentially no current, there are no whirlpool phenomena. In moving water, in rivers, marked eddies arise only in very specific parts of the stream: beyond underwater obstacles (submerged logs, trees, stumps, sunken vessel, huge rocks), on the boundary between two currents flowing at different velocities; beyond a rocky or jagged clayey projection from the shore, which is not subject to erosion, when the current presses the stream of water to it, beyond bridge piers. All such zones are rather rare and do not present a hazard to swimmers. Those exposed to them merely reported that the customary sensation of "obedient" water disappeared.

Vortical eddies, which do indeed pull in everything near them, may occasionally appear near hydraulic works when there is a difference in water levels on either side of the barrier, in the headwater before a GES [hydroelectric power station] spillway dam or in lock chamber, when water runs off through deep [bottom] catchment areas or culverts. However, this can be observed only at the construction stage, since one strives to avoid eddy formation, since the throughput capacity of the opening diminishes due to a gust of air into an eddy.

What should be done if a swimmer does find himself in a whirlpool region? As a rule, it can be seen from a distance and if the individual is not swimming at night or dusk, he can bypass it. When, however, it is inevitable to pass through a whirlpool, it is necessary first of all to remain calm, head directly toward it and swim energetically through this area, without stopping or dropping the legs. The fact of the matter is that, if he drops his legs, the swimmer inevitably assumes an erect position and virtually ceases to advance, and all his efforts are spent on "balancing" the head that is sticking out of water.

Since the extent of a whirlpool (with rare exceptions) is measurable in a few meters, it is sometimes sufficient to make 10-15 leg movements in spurts combined with coordinated arm movements to pass over it. One should not submerge under water or dive to bypass the epicenter at a greater depth, since one can lose one's bearings underwater or else hit the obstacle that generated the eddy. Moreover, for a swimmer it is psychologically important to feel in command of the situation at all times, rather than a victim of a disaster.

What does one need to know about cramps? Cramps are a motor disorder that is manifested by involuntary rhythmic or continuous muscular contraction. In the case of clonic seizures, the muscle contractions follow one another at short intervals, alternating with relaxation. Tonic seizures are contractions without relaxation. They may be associated with moderate and unpleasant tugging pain. They occur due to different causes, sometimes when there is severe and excessive physical tension. Only tonic seizures are observed when swimming.

It is believed that seizures can develop while swimming if an individual moves from warm water to a strip of cold water. We can agree that a drastic change of temperature is not pleasant while swimming, but there are no physiological grounds here for appearance of cramps. The opinion is also held that a cramp can be stopped by pricking the painfully contracted muscle, and for this reason some swimmers put a pin in their fins. It should be noted here that seizures occur in water in adults, rather than children who stay in the water until they turn blue and start to shiver. Perusal of the written accounts revealed the following pattern: seizures develop when an individual experiences fear or anxiety in the water, as well as following some spurt of swimming in the presence of psychogenic stress, etc.

In a study of this phenomenon, data were obtained that enable us to maintain that seizures occur while swimming on the principle of an ideomotor act, i.e., they result from mental self-induction. It has been established that people who have experienced a seizure when swimming somehow coped with it and reached the shore, then became convinced that a cramp is not a fatal phenomenon and were no longer afraid of it, remarking that it never recurred, no matter how long they had to swim in cold water and whatever physical exertion the swim required.

Many readers write that during the most intensive training in pools they did not experience seizures, but the moment they swam out in the sea or a river (at about the same water temperature) they developed a cramp. Now this is easy to explain: in a pool there is the subconscious thought that "the wall is nearby," whereas in open water, "who knows what could happen." These facts confirm once more the psychogenic nature of swimming cramps.

But what should be done if they occur anyway? In the first place, one should be calm about them. In the second place, since seizures appear in a group of flexor muscles one must make the opposite, extensor movement and relax the limb. In the third place, one should give oneself a massage. For this, having taken a breath and assumed the "float" position with the head in the water, one should rub and massage the muscles with both hands.

It is quite important to learn how to float in order to be confident under any conditions. Expressly static swimming enables the swimmer to rest on his way to shore, slow down his breathing and heart rate, relieve muscular fatigue and remain in water for an unlimited time, if necessary (of course, provided the temperature is appropriate). A deep inspiration renders the body buoyant and enables anyone to lie near the surface, in both fresh and salt water. There are no grounds for statements to the effect that this cannot be done by everyone, only some people; everyone can do the "float," "jellyfish" or "sun" exercises!

What are the physics of floating? Any free floating solid moves in fluid in such a way that its center of gravity is on the same vertical plane as the center of submerged volume. If this does not occur, the solid is in a state of unstable equilibrium and strives to correct it. The center of gravity of the human body is on the level of the 1st-2d sacral vertebrae, while the center of volume is a few centimeters closer to the head. For this reason,

in horizontal position with the arms along the trunk the legs want to sink. This lasts until the body assumes an almost vertical position and the center of gravity is below the center of volume. The legs also sink when the arms are outspread. This downward motion of the legs pulls the swimmer under water over his head, but the submersion can be stopped by paddling lightly with the legs as in the crawl or breast stroke, or "pedaling"; one can also execute thrusting motions with the arms although it is true that this already requires some exertion and precludes complete rest and immobility.

In order to provide stable horizontal equilibrium in a resting position one must, after taking a deep breath and holding it, like in water on one's back and bring the arms straight over the head so that they would be, so to speak, a continuation of the body. If the legs still sink in this position, one can stick the fingers or hands out of the water. This will automatically cause the legs to surface and the tips of the feet will surface. The entire body and legs may also be in a slightly tilted position under water. In order to rest, it is important to stay in a relaxed and motionless position, breathing freely.

Some people with high buoyancy place their arms, bent at the elbows, under the head and, lifting them slightly out of the water, control equilibrium. It is often sufficient to spread the arms out or spread the legs wide for the center of gravity to shift toward the head and then the legs surface.

The following mistakes may occur when one forgets the resting position: hands out too far from the water (not only the hands, but arms and elbows protruding from the water); the abdomen or chest is too far out of the water; the head is not tilted back enough due to fear that water will cover the face; incorrect breathing. Breathing must be done as follows: short and complete inspiration, hold the breath for a few seconds and exhale gradually, then the next deep inspiration without a pause.

Having learned to rest in shallow waters, one must also learn to do this when swimming to shore, turning from the abdomen to the back.

When swimming in windy weather, little-trained swimmers could choke on water and inhale it at the time of inspiration. Water in the larynx and trachea elicits a coughing attack and temporarily renders the swimmer unable to swim. In this case, if one must continue swimming in the face of a small breaker, one should turn away from it briefly. It is easier to cough and eliminate water from the respiratory tract if one immediately turns over on the back and, barely moving the legs, remains in this position until breathing is calm. Then it is desirable to swim on one's back or side to avoid a repetition of these dangerous occurrences.

The role of suggestion and autosuggestion is well-known. Everyone must have some skill in both. In situations in the water, it is important not only to know how to calm down an anxious comrade, instill him with faith in a good outcome, his own strength, but also how to "bring oneself to one's senses."

Skill, perfection and experience are acquired only by repeating any process many times. Swimming is no exception: the more one swims, particularly in open waters, the sooner one gains confidence, the more reliable the development and conditioning of mental and physiological reactions.

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## METHODS

UDC: 629.7.023.26

### METHOD OF CALCULATING ANGULAR DIMENSIONS OF FLIGHT VEHICLE COCKPIT CANOPY CASINGS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 18 Oct 83) pp 81-83

[Article by A. V. Lekarev]

[Text] The view from flight vehicle (FV) cockpits is an important factor in assuring flying safety and providing comfortable conditions for the pilot's visual observation of space outside the cockpit.

At the present time, transparent parts of most cockpits in different types of FV are executed in the form of flat panels installed into the metal frame of the cockpit canopy. Presence of opaque components (casings) in the cockpit canopy results in obscuring part of the outside area, which worsens viewing conditions from the pilot's work place.

In order to improve the view from FV cockpits with flat transparent parts, a method was developed for calculating the angular dimensions of casings [seams?] on the basis of distinctions of human binocular vision. The proposed method makes it possible to meet the requirements as to strength features of the canopy frame and provide for comfortable viewing conditions outside the cockpit. The method is based on crossed diplopia in an empirical horopter from a point of the cyclopic eye of the observer [1, 2]. Let us discuss the diagram illustrated in Figure 1.

Mathematically, the problem is to find the following functions:  $\vartheta = F_{\vartheta}(\alpha)$ ,  $\alpha = F_{\alpha}(\vartheta)$  and  $\varphi = F_{\varphi}(\alpha)$ .

To find these functions, let us express the following segments as certain variables:  $D$ --diameter of horopter,  $r$ --radius of horopter,  $d$ --distance between pupils,  $h$ --height of SQF segment,  $Q$ --point of cyclopic eye,  $a$ --distance to point of diplopia,  $l$ --magnitude of diplopia,  $\alpha$ --angle of rotation of axis of cyclopic eye,  $S$ --point on left eye and  $F$ --point on right eye.

When considering right-angled triangles  $GQN$  and  $LQK$ , we shall have:

$$GQ = D \cdot \cos \alpha, \quad (1)$$

$$LQ = \frac{h}{\cos \alpha}, \quad (2)$$

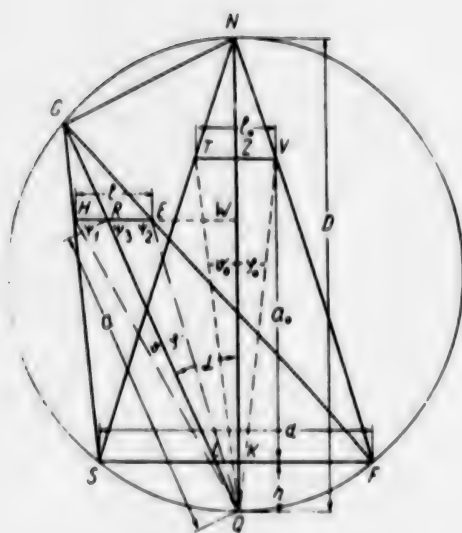


Figure 1.

Diagram of physiological crossed diplopia with binocular vision. Explanation is given in the text.

We then find  $RQ = a = F_a(\alpha)$ . For this purpose, from the similarity of triangles GRE and GLF we first find:

$$\frac{GR}{GL} = \frac{RE}{LF}. \quad (8)$$

Taking into consideration equations (3), (4) and (7), we find GR from equation (8):

$$GR = \frac{GL \cdot RE}{LF} = \frac{l}{d} \left( D \cos \alpha - \frac{h}{\cos \alpha} \right). \quad (9)$$

The presence of function (9) enables us to determine one of the sought functions,  $F_a(\alpha) = RQ$ :

$$\begin{aligned} a = F_a(\alpha) = RQ = GQ - GR = D \cos \alpha - \\ \frac{l}{d} \left( D \cos \alpha - \frac{h}{\cos \alpha} \right), a = F_a(\alpha) = \\ = D \cos \alpha \left( 1 - \frac{l}{d} \right) + \frac{lh}{d \cos \alpha}. \end{aligned} \quad (10)$$

In order to find sought functions  $E_\varphi(\alpha)$  and  $F_\varphi(\alpha)$ , we apply the theorem of sines of scalene triangles HQR and REQ:

$$\frac{HR}{\sin \psi} = \frac{RQ}{\sin \psi_1}. \quad (11)$$

$$LK = h \operatorname{tg} \alpha, \quad (3)$$

$$GL = GQ - LQ = D \cos \alpha - \frac{h}{\cos \alpha}, \quad (4)$$

The segments are  $TV \parallel HE \parallel SF$  by hypothesis. Consequently, the beams coming from point G separate on segments HE and SF proportionate segments:

$$\frac{HR}{RE} = \frac{SL}{LF}. \quad (5)$$

Substituting known values in equation (5) and considering that  $HR + RE = l$ , we obtain:

$$RE = \frac{l}{d} \left( h \operatorname{tg} \alpha + \frac{d}{2} \right), \quad (6)$$

$$HR = l \left( 1 - \frac{h \operatorname{tg} \alpha + \frac{d}{2}}{d} \right). \quad (7)$$

$$\frac{RE}{\sin \varphi} = \frac{RQ}{\sin \psi_2} \quad (12)$$

From right-angled triangles HWQ and RWQ we see that:

$$\psi_1 = 90 - (\alpha + \nu), \quad (13)$$

$$\begin{aligned} \psi_2 = 180 - \varphi - \psi_1 = 180 - \varphi - (90 - \alpha) = \\ = 90 + (\alpha - \varphi). \end{aligned} \quad (14)$$

With consideration of equations (13) and (14) we shall have:

$$\frac{HR}{\sin \nu} = \frac{RQ}{\sin [90 - (\alpha + \nu)]} = \frac{RQ}{\cos (\alpha + \nu)}, \quad (15)$$

$$\frac{RE}{\sin \varphi} = \frac{RQ}{\sin [90 + (\alpha - \varphi)]} = \frac{RQ}{\cos (\alpha - \varphi)}. \quad (16)$$

Solving equations (15) and (16) for  $\nu$  and  $\varphi$ , and substituting the expressions for HR and RQ, we obtain the sought functions  $F_\nu(\alpha)$  and  $F_\varphi(\alpha)$ :

$$\nu = \arccotg \left[ \frac{ad}{l \left( \frac{d}{2} - h \operatorname{tg} \alpha \right)} + \operatorname{tg} \alpha \right], \quad (17)$$

$$\varphi = \arccotg \left[ \frac{ad}{l \left( \frac{d}{2} + h \operatorname{tg} \alpha \right)} - \operatorname{tg} \alpha \right]. \quad (18)$$

Let us express height  $h$  of segment SQF in horopter parameters:

$$h = r - \sqrt{r^2 - \frac{d^2}{4}}. \quad (19)$$

Substituting equation (19) in (10), (17) and (18), we finally obtain:

$$\begin{aligned} a = D \cos \alpha \left( 1 - \frac{l}{d} \right) + \\ + \frac{l \left( r - \sqrt{r^2 - \frac{d^2}{4}} \right)}{d \cos \alpha}, \end{aligned} \quad (20)$$

$$\begin{aligned} \nu = \arccotg \left\{ \frac{ad}{l \left[ \frac{d}{2} - \left( r - \sqrt{r^2 - \frac{d^2}{4}} \right) \operatorname{tg} \alpha \right]} + \right. \\ \left. + \operatorname{tg} \alpha \right\}, \end{aligned} \quad (21)$$

$$\varphi = \arctg \left\{ \frac{ad}{\left| \frac{d}{2} \cdot \left( r - \sqrt{r^2 - \frac{d^2}{4}} \right) \lg \alpha \right| - \lg \alpha} \right\} \quad (22)$$

We calculate  $l$  on the basis of parameter  $\alpha$ . It is easy to find  $l$  from equation (20):

$$l = \frac{(D \cos \alpha - a) d \cos \alpha}{D \cos^2 \alpha \left( r - \sqrt{r^2 - \frac{d^2}{4}} \right)} \quad (23)$$

However, the designer is ultimately concerned with the allowable width of casing  $l_n$  at  $\alpha \neq 0$ , rather than with  $l$ . The value of  $l_n$  is determined graphically proceeding from the distinctions of cockpit shape at the site of the casing.

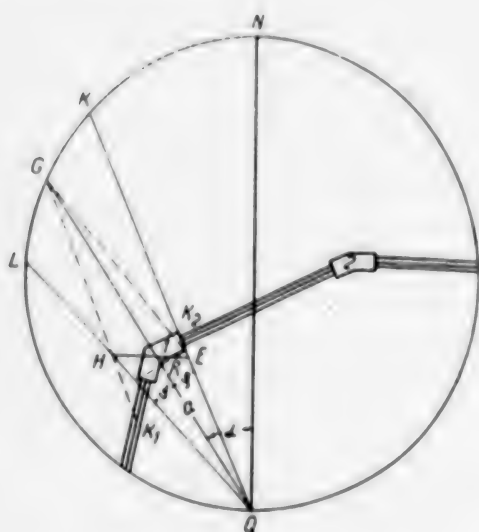


Figure 2.

Graphic diagram for determination of angular dimensions of cockpit canopy casings for commercial aircraft with a crew of two pilots (part of left side of cockpit). Explanation is given in the text.

point Q on line QN, with a radius  $r = 3$  m, on the established scale. One can also use a larger horopter radius, but then it must be borne in mind that the distance of missed binocular zone beyond the casing will increase accordingly. Then, from point Q, we draw line GQ through the center of the casing

Of course, determination of allowable angular dimensions of  $l_n$  is related to several factors that must be considered when designing cockpits (they include, for example, an adequate margin of safety of casings, their shape, distinctions as to securing transparent parts to the frame, angle of inclination of casings, etc.).

Let us discuss the procedure for determining  $l_n$  with  $\alpha \neq 0$ . We determine  $l_n$  graphically in the following order (Figure 2).

We plot on the graph on a scale that is convenient for calculations the outline of the cockpit canopy of the FV showing the places for tentative placement of casings 1, 2 and a point in the cyclopic eye of pilot Q (see Figure 2). We draw line QN from point Q parallel to the FV axis of symmetry. For the sake of simplicity, let us calculate the optimum angular dimensions of the casing only at point 1. We draw a circle through



projection point R, and measure on the diagram the values for  $\alpha$  and  $a = RQ$ . Knowing the values of D,  $\alpha$  and  $a$ , we use formula (23) to calculate  $l$  (according to the literature,  $d = 64$  mm [2]). Then, using formulas (21) and (22) we calculate  $\nu$  and  $\varphi$ , and draw lines QL and QK from point Q to the left and right of line QR. From point R, perpendicularly to QN, we draw a line to its intersection with QL and QK and obtain segment HE. We connect points H and E with dotted lines from point G and, continuing them to the intersection of the canopy outline at points  $K_1$  and  $K_2$ , we find the value of  $l_n$ . Analogously, we calculate the optimum angular dimensions of canopy casings installed in other parts of the FV cockpit.

The described method can be used not only at the designing stage, but to assess optimum angular dimensions of cockpit canopy casings in existing types of FV. In addition, it can also be used for cockpits with flat transparent sections in any type of transport and self-propelled machines, in which it is necessary to provide for the operator comfortable conditions for observing the exterior space.

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INFORMATIVENESS OF ECHO SIGNAL IN PULSED ULTRASONOGRAPHY OF THE BRAIN  
(WITH USE OF MODEL)

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19,  
No 4, Jul-Aug 85 (manuscript received 17 Jan 84) pp 83-85

[Article by L. G. Simonov, L. A. Rozenblyum and N. N. Bogdanova]

[Text] A change in hydrostatic pressure leads to change in intracranial volumetric relationships (IVR) of liquid media [3], which has a substantial effect on intracranial pressure and blood supply to the brain [1, 4]. As a noninvasive method of assessing IVR, one can use ultrasonic pulsed probing of the walls of the cerebral ventricles [6]. However, because of its low noise immunity and several other factors that affect the amplitude of the echo signal, the reliability of such a method for evaluation of IVR of liquid media is not satisfactory. We are dealing here with validation of a method of probing intracranial structures, in which the ultrasonic pulse penetrates through the frontal bone, passes through the ventricle and is reflected from the stationary occipital bone [5].

A model was developed to determine the informativeness of an echo obtained in this manner. It was demonstrated on this model that a change in level of the echo signal is elicited by change in ventricular volume. The base width of the ventricle causes this to be a monotone function. It was shown that such a probing method is less sensitive to change in acoustic properties of brain tissue than ultrasonic methods involving direct probing of ventricular walls.

#### Methods

In developing the model, we took into consideration the distinctions of functional and structural organization of the blood and spinal fluid circulatory system of the human brain, namely, existence of a sealed cavity containing the spinal fluid and blood systems, and brain tissue. A plastic skull is the basis of the model, and its shape and dimensions correspond to those of the human skull (Figure 1a). The "cover" of the skull is executed in plexiglas so that the intracranial space can be viewed. There is a clamping device on this cover which, with a liner, seals the "cranial cavity."

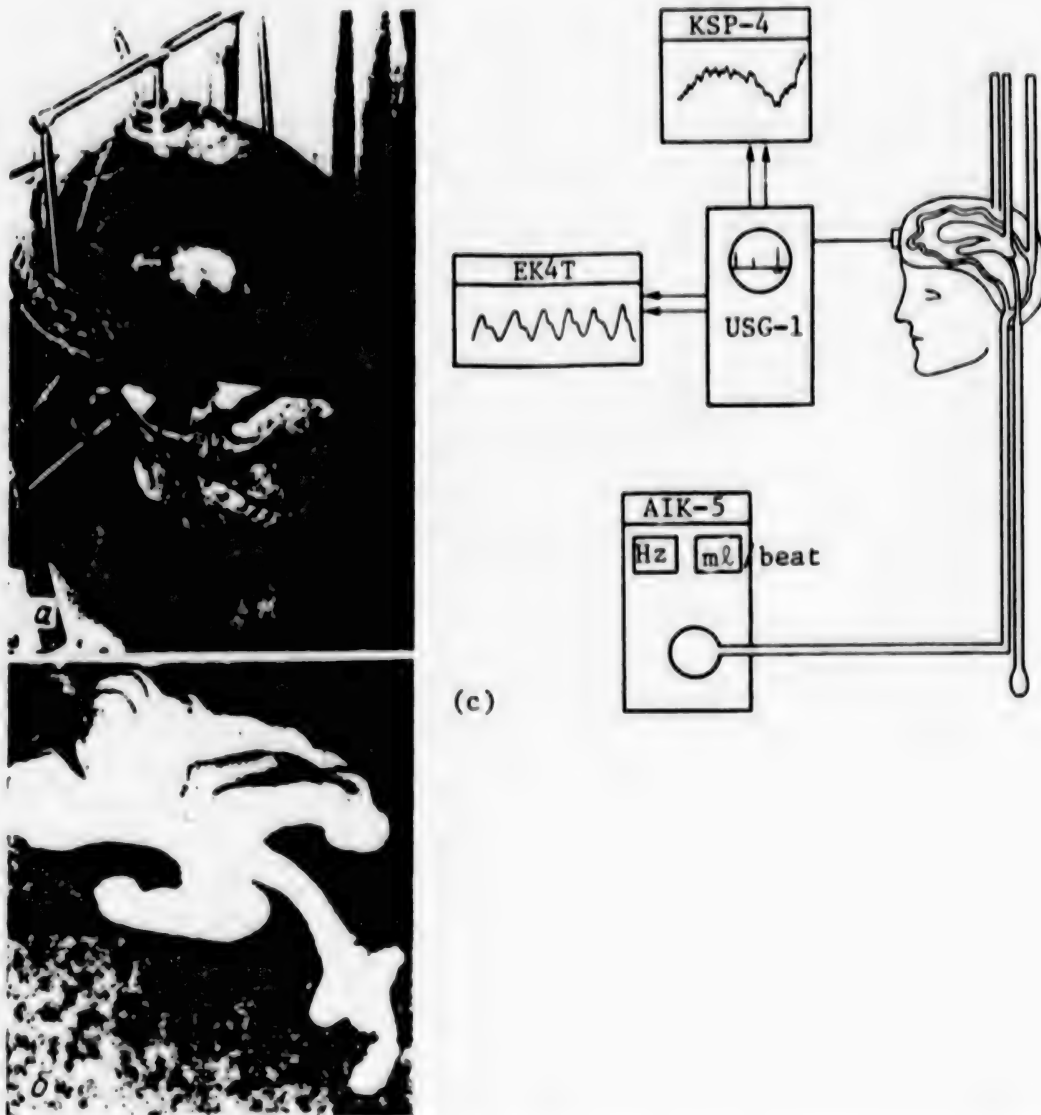


Figure 1. Model of human spinal fluid and blood circulatory system

- a) external view of model      c) flowcharts of device and model  
b) model of ventricles

The intracranial arterial system is represented by a flexible tube 0.2 cm in diameter and 180 cm in length, which is used for vascular prosthetics. Pulsation of pressure in it is generated by an extracorporeal circulation machine (AIK-5), which permits control of both amplitude of pulse pressure and frequency of its change. The spinal system is simulated with two communicating sections, intracranial and extracranial. The former, which simulates the cerebral ventricles, is made of latex and is as close as possible in shape to the ventricles of the human brain (Figure 1b). The second compartment consists of a rigid tube, at the end of which there is an expandable chamber (Figure 1c) that simulates the cerebrospinal "reserve spaces" [7]. The

inside diameter of the tube is 1.5 cm and its length is 60 cm, which corresponds approximately to the actual dimensions of the human cerebrospinal fluid canal. The compartments are interconnected with an adapter (0.2 cm in diameter), which simulates the foramen of Magendie. All systems in the model are filled with water. To reproduce effects related to redistribution of fluids caused by postural tests, the model can be secured on a holder that permits altering its spatial position if necessary.

To determine the informativeness of the echo reflected from the occipital bone, the ultrasound transformer was placed on the frontal bone of the skull in the model so as to have nothing other than the ventricle on the path of the ultrasonic beam.

Since the model studies described here are related to validation of the ultrasonic bioecholocation method [5], all of the methodological procedures pertaining to location of the sensor and choice of direction of probing were identical to those used in examining the dynamic characteristics of the spinal fluid and blood in the human cerebrospinal system.

We used a USG-1 instrument as the ultrasonic probe.

## Results and Discussion

The level of the echo signal changed with change in volume of the ventricle, and the monotone nature of this function was determined by the base width of the ventricle. When the ventricle is wide and overlaps a significant part of the ultrasonic beam, the increase in ventricular volume leads to increase of echo level. If, however, the ventricle is narrow and overlaps an insignificant part of the ultrasonic beam, an increase in ventricular volume leads to decrease in level of the echo signal. In the intermediate case, with growth of ventricular volume the echo signal first decreases and then increases.

After connecting the extracorporeal circulation machine (AIK-5) pulsations were generated in the cranial cavity, which led to pulsating movements of the ventricles. The pulsation of the echo they induced in the presence of a wide ventricle occurred in antiphase with change in pulsed pressure; in the case of a narrow ventricle, it occurred synphasically and in the intermediate case, we observed change from synphasic to antiphase pulsations with increase in ventricular volume. Consequently, the series of ultrasonic pulses that passed through a structure with variable volume acquired amplitude modulation, the form of which depends on the ratio of volumes to area of emitting surface.

Theoretically, the change in acoustic properties of brain tissue elicited by pulsed delivery of blood to brain vessels could change the type of modulation of the echo signal that has passed through the ventricle and is reflected from the occipital bone.

The following tests were performed to compare the sensitivity of this method of monitoring ventricular volume to widely used methods of direct probing of the walls of the third ventricle, with regard to change in acoustic properties of brain tissue. Settled tap water, which simulated nonexpandable brain tissues was poured into a rectangular plexiglas aquarium. The latex ventricle,



also filled with tap water, was immersed in it. The ultrasound emitter was so placed as to have the ultrasonic beam overlap the ventricle, after which we assessed the level of the echo reflected from the anterior wall of the ventricle and posterior wall of the tank. We then added table salt to the water in the tank to obtain a 5% change in acoustic resistance of the medium surrounding the ventricle and monitored relative change in level of the analyzed echo signals (Figure 2). From the plot we see that the echo

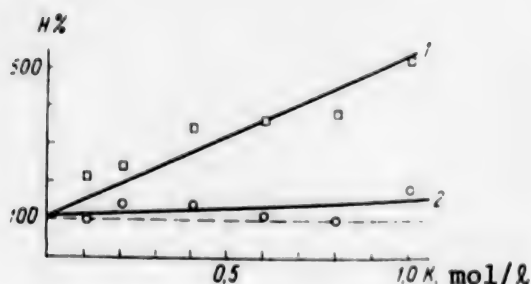


Figure 2.

Relative change in level of echo signals with change in acoustic resistance of medium

- 1) signal from anterior wall of ventricle
- 2) signal from posterior wall of tank

the posterior wall of the skull is related to change in ventricular volume. The monotone nature of this function is determined by the initial width of the ventricle.

signal reflected from the anterior wall of the ventricle undergoes much greater changes than the one that traversed the ventricle and was reflected from the wall of the tank. In order to determine whether the obtained effect is a property of a complex interface (solution--latex--water) or whether is solution--latex boundary is analogous to the brain tissue--spinal fluid boundary, we repeated the test with a thick ventricle ( $H = 2.5$  mm). There was no change in qualitative findings in this case.

In the first series of tests performed on the model, it was established that a change in level of the echo from

The results of the second series of tests conducted in the tank revealed that a change in acoustic properties in the ventricle's environment (salt solution for model) has a substantially greater effect on the level of the echo reflected from the ventricle than on the signal that passed through the ventricle and was reflected from the posterior wall of the tank. One can comprehend such behavior of echo signals on the basis of simple theoretical considerations. As we know [2], the coefficient of reflection from the interface of two media with acoustic resistance  $\rho_2 C_2$  and  $\rho_1 C_1$  ( $\rho$  is density of material and  $C$  is velocity of ultrasound) is proportionate to the ratio:

$$\frac{\rho_2 C_2 - \rho_1 C_1}{\rho_2 C_2 + \rho_1 C_1}$$

and the transmission coefficient, to  $\frac{2\rho_2 C_2}{\rho_2 C_2 + \rho_1 C_1}$ . For this reason, the signal

reflected from the tank wall that crosses through the ventricle twice is proportionate to:

$$\frac{(\rho_2 C_2)^2 (\rho_1 C_1)^2}{(\rho_1 C_1 + \rho_2 C_2)^4} \times \frac{\rho_3 C_3 - \rho_1 C_1}{\rho_3 C_3 + \rho_1 C_1}$$

where  $\rho_3 C_3$  is acoustic resistance of the tank wall (plexiglas). When there is minor relative change in  $\Delta \rho_1 C_1$  due to change in salt concentration, the relative change in signal reflected from the ventricle is proportionate to

$$\frac{\Delta(\rho_1 C_1) \rho_1 C_1}{\rho_2 C_2 - \rho_1 C_1}$$

whereas the echo from the tank wall undergoes changes proportionate to

$$\frac{\Delta(\rho_1 C_1) \rho_1 C_1}{\rho_3 C_3 - \rho_1 C_1}$$

Since acoustic resistance of latex is substantially closer to resistance of water than acoustic resistance of plexiglas, the relative change in level of echo reflected from the ventricle will be greater than for the signal reflected from the plexiglas wall. Since acoustic resistance of brain tissue and spinal fluid is similar ( $\frac{\Delta \rho C}{\rho C} \approx 0.01$ ) when probing the cerebral ventricles in vivo and resistance of the occipital bone is substantially higher, with change in acoustic properties of the brain due to change in delivery of blood to its tissues the echo reflected from the occipital bone does not undergo noticeable change, whereas the one reflected from the ventricular wall would change appreciably.

As a result of these model studies, it was shown that a change in intensity of the echo signal is related to change in ventricular volume. The obtained data permit more accurate interpretation of the results of in vivo tests that are performed to assess the intracranial volumetric relationships of fluids in man.

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INVESTIGATION OF POSSIBILITY OF USING TWO-FREQUENCY IMPEDOMETRY FOR  
ESTIMATION OF PROPORTION OF TOTAL AND EXTRACELLULAR BODY FLUIDS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19,  
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[Article by V. P. Krotov, Ye. G. Bazunova and B. S. Kulayev]

[Text] According to the results of postflight examination of cosmonauts, exposure to weightlessness leads to changes in fluid-electrolyte metabolism and in ratio between liquid phases of the body [4, 9].

It is not possible to establish the kinetics of fluid redistribution in the body as a function of duration of weightlessness, since a method has not yet been found that would permit measurement of body fluids and proportion of each in flight.

Recently, works have been published, which report that it is possible to determine the ratio of total body fluid to its extracellular component [2, 6]. This is done by concurrently determination of impedance ( $Z$ ) in the measured section of the body during passage of current through it at different frequencies.

$Z_1/Z_{100} = 1.30 \pm 0.03$  for current at frequencies of 1 and 100 kHz and  $1.50 \pm 0.05$ , at frequencies of 5 kHz and 1 mHz. In healthy man, the ratio between these two parameters ( $Z_{LF}/Z_{HF}$ ) is a constant for given frequencies of probing current. With age, the ratio diminishes. It also diminishes in the presence of virtually all forms of pathology where the process is generalized [3].

However, all of the studies cited above have a clinical and physiological orientation, with which it is impossible to determine distinctly the quantitative relationship between total fluid and its extracellular component.

Our objective here was to explore the possibility of using two-frequency impedometry to determine the quantitative ratio between total body fluid and its extracellular component.

#### Methods

The study was conducted in four series of experiments on rats. In the first series (11 animals), we examined the dynamics of change in  $Z$  of tissues in



the upper and lower halves of the trunk with exposure to orthostatic and anti-orthostatic perturbing factors. We changed the position of the body almost instantaneously; the tilt angles constituted +90 and -90°. Measurements were taken for the first 4-5 s, then 5, 10 and 15 min after changing position. The second series of experiments (13 animals) involved infusion of saline or polyglucin into the rat's right pleural cavity through a vascular catheter 0.7 mm in diameter, introduced in advance. In the third series (14 animals), we infused a strictly metered amount of saline or polyglucin through a catheter in the abdominal cavity. In the fourth series (8 animals) we measured the Z segment of the abdominal region with infusion of saline into the large intestine.

During the experiment, the animals were on a temperature-controlled table; the rats' body temperature did not change by more than 0.5°C. The solutions were infused at the animal's body temperature. Infusion of solutions increased primarily the amount of extracellular fluid, altering the ratio between total fluid and its extracellular component in the tested segment of the body.

The active and potential electrodes were introduced under the skin; the former around the extremities and the latter around the neck, 0.5 and 1.5 cm below the xiphoid process and on the level of the iliac crests. An experimental variant of the RPG-204 rheoplethysmograph was used as source of probing current, resistance at 2 frequencies, 1 and 100 kHz, was measured using a V 7-21 digital voltmeter. Margin of error in measuring Z did not exceed 2% at both frequencies.

The data were submitted to variation statistical processing.

## Results and Discussion

The dynamics of change in Z of the thorax in rats during perturbing postural changes at different frequencies of probing current and the proportion between these the 2 values are illustrated in Figure 1 (as percentage of base values). For the first few seconds after moving the body from horizontal to vertical position, thoracic Z increased by a mean of 7% ( $P < 0.01$ ). The increment constituted 3% ( $P < 0.05$ ) after 5 min and only 1% in the next 10 min. Returning the animal to horizontal position first led to marked change in Z of the tested region, and it decreased by 4-5% ( $P < 0.02$ ). In the next 15 min, the changes did not exceed 1%. In antiorthostatic position, thoracic Z changed less: it decreased by 3-2.5% ( $P > 0.05$ ) immediately after the position change and did not change thereafter. Return to horizontal position led to only 2.0-2.5% increase ( $P > 0.05$ ) in Z.

It should be noted that, regardless of frequency of probing current, thoracic Z changed to the same extent. For this reason, the value of  $Z_1/Z_{100}$ , which constituted 1.32 in the baseline period, did not change reliably throughout the experiment ( $P < 0.5$ ).

The dynamics of Z for the abdominal segment in rats submitted to postural changes are illustrated in Figure 2. Changing the animal to orthostatic position elicited only 1-1.5% ( $P > 0.05$ ) decline of Z, and in the 10-15 min that the animal remained in this position Z rose gradually, reaching the baseline value. When rats were changed to horizontal position, efflux of fluid from the tested segment of the abdominal region continued, causing Z to increase by

5% in 15 min ( $P < 0.02$ ). For the first few seconds of antiorthostatic position, Z rose by 5% ( $P < 0.02$ ) and by only 3% in the next 15 min ( $P > 0.05$ ). Returning the animal to horizontal position was associated with a decline of Z, which was the most marked immediately after the position change (by 4-5%).

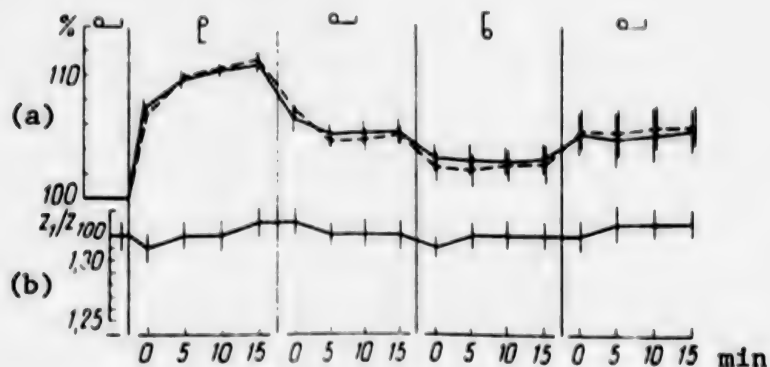


Figure 1. Dynamics of Z for rat thorax with changes in position

Here and in Figure 2: x-axis, time of recording Z in each period of postural changes (min); y-axis: a) relative changes in Z at the 2 frequencies during position changes (%); Z in horizontal position was taken as 100%; b) dynamics of  $Z_1/Z_{100}$  during postural changes. Dotted and dash lines--frequency of probing current, 1 and 100 kHz, respectively

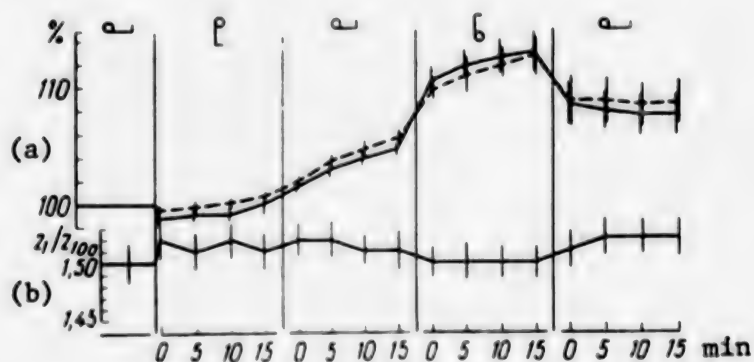


Figure 2. Dynamics of Z for lower half of rat trunk with changes in position

The changes in Z for the measured abdominal segment were the same, as in the chest, regardless of probing current frequency. For this reason,  $Z_1/Z_{100}$  equaled 1.50 in the baseline period and did not change reliably ( $P < 0.5$ ) at any stage of the functional test.

During the disturbing postural changes, when body fluids shift there can also be a change in intracellular component, so that we deliberately altered only the amount of extracellular fluid in the next three series of experiments. In

the second series this was obtained by infusing saline at the animal's body temperature into the right pleural cavity. With infusion of 1 ml saline,  $Z$  of the chest decreased by a mean of 2.5%. No reliable differences were demonstrable in nature of curves of  $Z$  changes at either 1 or 100 kHz frequency. Use of polyglucin was associated with analogous dynamics of changes in  $Z$  of the thorax, approximately 3% decline per ml infused agent. As in the case of saline,  $Z$  of the chest changed to the same extent, regardless of frequency of probing current.

In the 3d series, we determined  $Z_1/Z_{100}$  in the abdominal region into which warm saline was infused through a catheter (1 ml at 1-2 min intervals). As a result  $Z$  in this region changed to the same extent regardless of frequency of probing current, by about 1.5%/ml infused solution. Use of polyglucin was associated with a similar effect: regardless of probing current frequency,  $Z$  in the measured segment decreased by 1.5-2% with infusion of 1 ml of the agent (Figure 3).

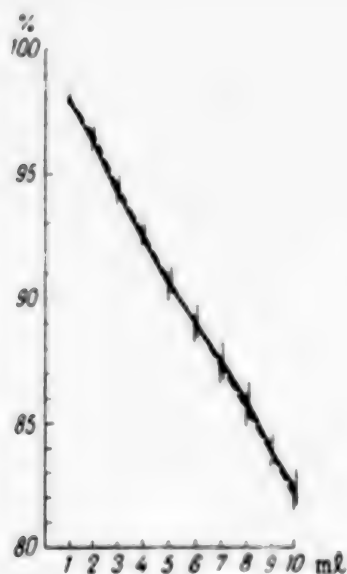


Figure 3.

Change in  $Z$  of lower half of rat trunk with infusion of polyglucin into the abdominal cavity

X-axis, amount of infused fluid (ml);  
y-axis, relative changes in  $Z$  at frequencies of 1 kHz (solid line) and 100 kHz (dash line) ( $Z$  prior to infusion was taken as 100%)

structure, i.e., sequence of tissues with capacitive and active properties, and dependence of electrical properties of tissue on frequency [1, 7]. The most significant capacitive component of  $Z$  due to capacitive resistance of the integument was excluded by passing electrodes under the skin. However, in order to rule out the possibility of dependence of  $Z_1/Z_{100}$  on conditions of electrode contact with tissue, in some of the experiments we changed the area of active and potential electrodes by more than 10 times. But even in this case,  $Z_1/Z_{100}$  did not change with infusion of fluid into the tested region.

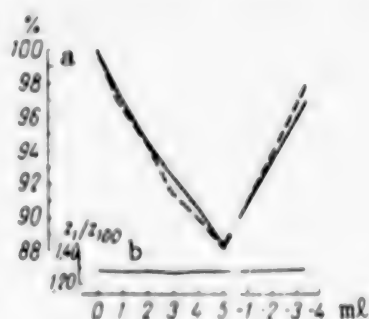


Figure 4.

Change in  $Z$  of abdominal segment in rat with infusion of polyglucin into the rectum

X-axis, amount of fluid infused and withdrawn (ml); y-axis:

- relative change in  $Z$  at two frequencies, 1 kHz (dash line) and 100 kHz (solid line) (value of  $Z$  prior to polyglucin infusion taken as 100%)
- dynamics of  $Z_1/Z_{100}$  ratio during experiment

It is known that  $Z$  of the examined part of the body as a function of frequency of probing current is determined primarily by two factors: its

When fluid is infused in the body's natural cavities, it can be localized in an isolated area that does not intersect the planes formed by the potential electrodes placed in parallel. Such heterogeneity of the tested body area could have affected the nature of change in  $Z_1/Z_{100}$ . For this reason, in the fourth series of experiments, we infused saline into the rectum, and the top potential electrode was placed deliberately below the transverse colon. In this case, both infusion and withdrawal of fluid led to the same change in  $Z$  at both frequencies (Figure 4). As a result,  $Z_1/Z_{100}$  remained unchanged.

A change from horizontal to vertical position leads to shifting of blood and part of the extracellular fluid [5]. Some amount is deposited in the abdominal (in orthostatic position) or thoracic (antiorthostatic position) regions [8]. As a result, there is a change in ratio between extracellular fluid and total body fluid, and consequently in the  $Z_1/Z_{100}$  ratio. In our experiments with postural factors,  $Z_1/Z_{100}$  remained unchanged in all cases. However, with change in position of the body in the earth's gravity field, there is also change in correlation between organs, in particular, the position of the diaphragmatic dome, heart, liver and intestine. As a result of shifting of organs in the examined segments of the thoracic and abdominal cavities, there is change in weight of probed tissue, and these changes may be significant, as compared to the displaced extracellular fluid. For this reason, we cannot rule out the probability of a phenomenon where the ratio between total and extracellular fluid remains virtually unchanged in the regions we tested with use of postural changes.

We ruled out the possibility of change in tissue mass in the tested segment of the body in series of experiments with infusion of strictly metered amounts of saline and polyglucin. But in this case too, the changes in  $Z$  at the frequencies we used were the same, i.e., there were no changes in  $Z_1/Z_{100}$  although there was noticeable increase in share of the extracellular component in the overall level of hydration.

Consequently, our studies failed to demonstrate a relationship between value of  $Z_1/Z_{100}$  and proportion of total fluid and its extracellular component in the tested segment of the body. Apparently, such a function could be established in the case of measurement of only the active component when the role of the capacitive component, which has low immunity to interference with measurements at frequencies of up to 5 kHz, rather than with measurement of impedance, i.e., total resistance of the segment.

At the same time, in our studies the  $Z_1/Z_{100}$  ratio was markedly distinct from 1 in all cases. With consideration of the segment of probed tissue, this ratio ranged from 1.32 to 1.50, i.e.,  $Z$  of the tissue segment depends on the frequency of current passing through it. The logical question arises as to the cause of difference in resistance of tissue segments when probing it with current of different frequencies if the changes in the body's liquid phases do not alter the base values of  $Z_1/Z_{100}$ . We cannot rule out the possibility that the tissue cell membrane is permeable to probing current at both frequencies, but it has 1.3-1.5 times more resistance to a frequency of 1 kHz due to its capacitive component.



Thus, the results of studies conducted with animals (postural tests, infusion of saline or polyglucin [dextran] into the pleural and abdominal cavities, as well as large intestine) do not confirm the findings of some authors [2, 3, 6] to the effect that it is possible to use two-frequency impedometry to examine redistribution of fluid in the body.

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AUTOMATIC DETERMINATION OF CARDIAC OUTPUT FROM RHEOGRAM OF THE TRUNK

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 28 Oct 83) pp 89-90

[Article by I. V. Sokolova and L. A. Khryashcheva]

[Text] Depending on the method of recording the trunk rheogram (TRG) and theoretical validation of formulas for subsequent calculations, many rheographic analogues of characteristics of cardiac output are calculated from the amplitude and period of the TRG with use of appropriate multiplicative coefficients. The technical execution of the system for automatic determination of characteristics of cardiac output on the TRG in the form of an algorithm for a computer does not impose restrictions on structure and number of coefficients, and it offers broad research opportunities for studying them in comparison of rheography to other methods.

The algorithm for automatic determination of cardiac output features from the TRG is executed in the form of a set of programs in the FORTRAN-IV algorithmic language for YeS or SM series computers, and it solves the following problems:

- 1) Isolation of rheowave as the valid signal in the presence of interference.
- 2) Identification of elements in the rheowave structure for the duration of the cardiac cycle.
- 3) Calculation of rheographic characteristics of cardiac output for the cardiac cycle.
- 4) Statistical averaging of rheographic characteristics of cardiac output in a specified interval for a specified level of significance.

Isolation of the rheowave as the useful signal in the presence of interference is a problem of theory of pattern recognition which, in this case, is solved on the principle of composing a description of rheowave properties that is necessary and sufficient for error-free separation from interferences. The baseline properties of the rheogram as an analogue signal for composing such a description are the working range of the spectrum and periodicity.

The working range of the rheosignal spectrum in the interval of 0-10 Hz defines it as a narrow-band low-frequency signal. The chosen interval for

quantization of rheogram ordinates for time, 15 ms, is 6.5 times greater than the top frequency of the spectrum and provides for rather high precision in calculating the TRG amplitude and period. Periodic interference in the range exceeding 10 Hz is eliminated by means of a digital filter at the appropriate cut-off frequency.

Periodicity of the rheowave is used to eliminate aperiodic interference on the order of drastic shift or significant fluctuations of isoline level, artefacts arising when the examined object moves, calibration, turning the rheograph on and off, etc. Periodic interference is eliminated with exclusion from further consideration of the segment of the rheogram where the noise was discovered. For this purpose, the analyzed segment of the rheogram is tested for periodicity of signal. That periodicity conditions are met is tested in the form of successive comparison to the appropriate allowances for the scatter of the following parameters: values for two adjacent absolute maximums of the first rheogram derivative for time (the rheowave period is isolated on their basis); maximums of rheograms for two adjacent periods; rheogram minimums for two adjacent periods; modulus of difference between period duration calculated from the first derivative and duration of the same period calculated from the absolute maximums of the second rheogram derivative for time; values for rheogram ordinates at the points corresponding to period start and end positions.

Failure to abide by any of these allowances is interpreted as the influence of interference, on the basis of which the analyzed segment of the rheosignal is excluded from consideration, a shift is made in the run for duration in one cardiocycle period to the next fragment and it is subsequently checked in a similar manner. Time differentiation is effected by means of a digital differentiating filter with smoothing by the least squares method and approximation of the rheogram to a quadratic parabola.

If all conditions for periodicity of the rheosignal are met, it means that the rheowave period has been isolated in a given segment and further processing can be done, i.e., identification of elements of its structure. The elements of rheowave structure for the period of the cardiocycle are the ordinates and points corresponding to current period rheogram start and end, the duration of this period (T) and amplitude of the TRG (A). Identification of structural elements of the rheowave is both the starting and ending condition for forming the specifications for performance ["noise immunity"] of the algorithm. For this reason, virtually all elements (with the exception of amplitude A) of the rheowave structure for the current period are determined already in the course of identification of the TRG signal.

At each period of the rheowave, which was isolated and for which the basic structural elements were identified, immediate values are formed for the following characteristics of cardiac output: TRG amplitude (A in ohms), pulse rate (PR/min), volumetric blood flow rate (FC in  $\Omega$ /s).

A (in  $\Omega$ ) is calculated using the following formula:

$$A = A_{ch}/E \quad (1)$$

where  $A_{ch}$  is maximum value of TRG ordinates in the period studied in relation to its ordinate at the starting point of the rheowave for this period

measured in V, E is calibration signal (in V/ $\Omega$ ) for conversion of TRG ordinates (in V) to their standard dimension (in  $\Omega$ ).

The immediate PR (per min) is calculated using the formula:

$$PR = 60/T \quad (2)$$

where T is duration of period (in seconds).

The rate of volumetric blood flow determined from the TRG, FC (in  $\Omega/s$ ) is calculated using the formula:

$$FC = A/T \quad (3)$$

This parameter is included, as an intermediate term, in the formula for determination of relative volumetric pulse VP (per thousand), which is calculated [1] in the following manner:

$$VP, \% = A \cdot 1000 / (R \cdot T) = FC \cdot 1000 / R \quad (4)$$

where R is basic interelectrode ohmic resistance of the area examined (in  $\Omega$ ). Resistance R forms the level of the isoelectric line of the TRG, and its slow change in time permits consideration of R as a parametric experimental constant.

On the other hand, volumetric blood flow rate FC, determined from the TRG, is a simplified variant of the more general expression for velocity of volumetric blood flow F (in  $\Omega$ ), which is determined from the rheogram of any part of the vascular system that is calculated [2] with the following formula:

$$F = (A + B)/T \quad (5)$$

where A is amplitude of the arterial component of the rheogram (in  $\Omega$ ) and B is maximum systolic value of venous component of the rheogram (in  $\Omega$ ).

The arterial and venous components of the rheogram reflect volumetric pulsed fluctuations of delivery of blood in the arterial and venous compartments, respectively, of the tested vascular region, which are related to propagation of the pulsed wave of blood and occur in relation to intrinsic constant components of interelectrode ohmic resistance that are components of R.

As a rule, for the TRG (unlike rheograms of other vascular regions), the amplitude of the arterial component coincides virtually always with the TRG amplitude, while the maximum systolic value of the venous component is low enough to be disregarded. If we also consider that it is virtually impossible to isolate the constant elements of arterial and venous components of the TRG in resistance R that forms the isoline level, the velocity of volumetric blood flow FC determined from the TRG is a rather interesting independent feature of cardiac output.

Statistical averaging of rheographic characteristics of cardiac output is done for a 45-s interval and 0.1 level of significance. The values for the calibration signal E (V/ $\Omega$ ) and ohmic resistance R ( $\Omega$ ) are given in the form

of parametric experimental constants. The results of automatic TRG processing are written up in accordance with the appropriate protocol in the form of a table indicating the date of the experiment, its code name, surname of subject and digital values of rheographic characteristics of cardiac output determined from the TRG. Operation of the system has been tested in space investigations.

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## BRIEF REPORTS

UDC: 629.78:574.682]:612.867

### FUNCTIONAL STATE OF THE OLFACTORY ANALYZER IN A PRESSURIZED ENVIRONMENT

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 10 Apr 84) pp 90-92

[Article by S. S. Pashin and G. I. Solomin]

[Text] Man's long-term presence in pressurized environments (PE) imposes certain requirements of the composition of the artificial atmosphere in space vehicles. It is important for there to be no odors of various impurities. The latter is attributable to the range of reflex effects of chemicals through receptors of the upper respiratory tract on other analyzers, functional state of the central nervous system, various organs, systems and the body as a whole. Thus, some compounds elicit a number of reflex effects when introduced into the upper respiratory tract, primarily a trigeminal effect that is manifested by inhibition of respiratory center function, slowing of heart rate and blood pressure drop [3]. Experiments involving stimulation and successive deafferentation of the isolated upper respiratory tract revealed that it is of deciding significance in such reactions [5]. The purely olfactory effect of chemicals can also be an adverse factor. A. I. Bronshteyn [1], who tested the effect of brief exposure to various odorous stimuli on change in functional lability of nervous processes in the cerebral cortex, discovered that inhalation of fumes of compounds, the odor of which is subjectively perceived as unpleasant, diminishes functional lability and this affects man's work capacity. Odorous stimuli can be the cause of development of severe headache, vertigo and nausea. Low concentrations of odorous agents in the air could, if exposure is prolonged, elicit accentuation of sensitivity to them and lead to intolerance to these odors. In some cases, the sensitizing nature of the effect can be demonstrated even with short-term (5-15 min) inhalation of air containing odorous agents [4].

It is particularly undesirable to have persistent odorous stimuli in the atmosphere of a pressurized environment, and for this reason odorimetric studies are a mandatory stage of setting hygienic standards. However, such studies are performed under ordinary laboratory conditions, without consideration of the possible functional changes in the olfactory analyzer related to long-term isolation of an individual. Yet information of this kind, which has not been reflected in the literature, could be of great practical value for correction of hygienic standards as they apply to PE. Our objective here was to demonstrate any possible functional changes in the olfactory analyzer during long-term exposure of man to the artificial atmosphere of a pressurized chamber.

## Methods

This study was conducted on 3 subjects who spent 3 months in a pressure chamber with normal levels of the main components of air. The functional state of the olfactory analyzer was assessed by the threshold of the odor of the tested agent in each subject at different stages of their stay in an artificial atmosphere. Odorimetric tests were performed at 2-week intervals in order to rule out any possible factors of adaptation and sensitization of the analyzer. Pressure chamber conditions did not enable us to use the traditional approaches to evaluation of the threshold of olfactory sensation, and for this reason we used a modification of known static methods [2].

We first produced specific concentrations of the tested agent in Zeger pipettes (200 ml). Using a silicon tube, the subject alternately connected each of the pipettes to bulbs introduced in the nostrils to form a seal, opened both valves of the pipette and performed a forced inspiration (smelling motion). In order to provide the required concentrations in the pipettes, a microbatch of the tested agent was put in a desiccator with a magnetic mixer. After evaporation of the agent from the desiccator a syringe was used to collect an air sample of a specific volume, which was determined using the following formula, depending on the required concentration:

$$V_1 = \frac{V_2 \cdot V_3 \cdot C}{m}$$

where  $V_1$ ,  $V_2$ ,  $V_3$  are volumes of the syringe, pipette and desiccator (in l), respectively,  $C$  is the required concentration (mg/l) and  $m$  is the batch of agent (mg). This volume of air, which contained fumes of the tested agent, was transferred from the syringe to the pipette. Air samples were examined by a chromatographic method to monitor concentration levels.

To assure introduction of the agent in a leak-proof manner into the nasal cavity, we individually fitted the bulbs for each subject; the bulbs consisted of an plexiglas attachment connected to a glass three-way tube. Each of the subjects was given 9 pipettes with different amounts of the tested agent, and 4 of them were filled with pure air, serving as a control.

We selected butyl acetate as the tested agent, since it has been identified the most often among the gas emissions from nonmetal materials used to outfit PE. This was confirmed by the results of sanitary and chemical tests of the atmosphere of a mock-up of the Salyut-6 orbital space station [7]. The results of preliminary chemical tests revealed that the concentration of butyl acetate was close to nominal.

## Results and Discussion

The results of odorimetric tests enabled us to demonstrate that there is the same direction of changes in acuity of olfaction in the subjects, while there were individual differences. As can be seen in the Table, the threshold of butyl acetate odor dropped on the average from 0.02 to 0.002 mg/l during the stay in the chamber, i.e., to 1/10th the baseline. This is indicative of significant intensification of sensibility of the olfactory analyzer to presence of butyl acetate in inspired air as early as 1 month after the start of the study. Thereafter, sensitivity of the olfactory analyzer showed virtually no change, and the threshold for butyl acetate odor held at close to 0.002 mg/l.

Apparently, the demonstrated heightening of sensitivity of the olfactory analyzer is a reaction to the effect of long-term isolation.

Change in threshold for odor of butyl acetate (mg/l) in subjects at different stages of stay in pressure chamber

Subject	Baseline	Day of observation				
		14	21	28	44	70
L-n	0,029	0,016	0,012	0,0043	0,0018	0,0068
K-ch	0,016	0,0032	0,0012	0,00034	0,00018	0,00015
M-v	0,016	0,0032	0,0014	0,00086	0,0005	0,00053
$\bar{X}$	0,02	0,0075	0,0049	0,0018	0,00083	0,0025
$\pm\sigma$	0,0043	0,0043	0,0029	0,0012	0,0005	0,0013
$P$	-	>0,05	<0,05	<0,05	<0,05	<0,05

There is information in the literature to the effect that general functional level of the central nervous system declined in experimental animals, not only during total but relative sensory isolation. Even insignificant changes in the stereotype of conditioned stimuli could lead to impairment of higher nervous activity [8].

The artificial atmosphere in a PE can be viewed as a factor of sensory isolation of the olfactory analyzer, since the air in closed environments is virtually wanting in the customary range of odorous stimuli but can contain trace impurities that are recorded by the olfactory receptors relatively seldom under ordinary conditions. For this reason, the changes we demonstrated in these studies may be a compensatory and adaptive reaction to this factor. On the other hand, the demonstrated effect could be related to change in general functional state of the central nervous system. An analogous situation prevailed in a study of the effect on man of social isolation and sensory deprivation [9], as well as of constant monitoring by the researcher of the subject when the latter was totally isolated [6]. In addition, it should be noted that an individual in a closed environment, which does not exclude the probability of an emergency situation, is under some psychological stress, for which reason the olfactory analyzer acquires special significance as a rather sensitive apparatus that permits rapid registration of changes in gas composition of the artificial atmosphere and can prevent undesirable consequences. For this reason, olfaction may be heightened.

The findings make it necessary to consider not only the intensity of odor of chemicals that are subject to hygienic standards, but change in sensitivity of the olfactory analyzer to these compounds. The changes may be selective, and for this reason correction of maximum allowable concentrations may vary for different agents.

Consequently, odorimetric studies should be one of the stages in setting hygienic standards for chemicals in the air of PE. We cannot rule out the possibility of compensation of changes in olfactory analyzer function by means of certain preventive agents. Thus, if we consider sensory isolation

of the analyzer to be the main factor affecting olfactory function, addition to the artificial atmosphere of a set of chemicals (mainly of natural origin), which do not form a persistent odor, may be such a preventive measure. One should also take into consideration the individual distinctions of a person, his subjective perception and objective signs. Such measures require special investigations in order to determine scientifically validated and optimum parameters for composition of an artificial atmosphere.

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GAS CHROMATOGRAPHIC ANALYSIS OF FREE FATTY ACIDS OF SKIN SURFACE LIPIDS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 19, No 4, Jul-Aug 85 (manuscript received 2 Apr 84) pp 93-94

[Article by V. P. Naydina, B. L. Avetisyan and D. M. Dubinin]

[Text] The basic components of sebaceous gland secretions are triglycerides, which are hydrolyzed to free fatty acids, monoglycerides and diglycerides on the skin surface, under the effect of lipases of microorganisms and other factors [8]. Studies of the microflora of the skin surface revealed that it contains aerobic staphylococci, anaerobic propionobacteria and fungi which have lypolytic activity [4, 6]. A correlation was established between quantity of microorganisms of the micrococcus family and acne propionobacteria on the skin, on the one hand, and rate of formation of free fatty acids (FFA) of sebum cutaneum, on the other hand [2].

The bactericidal and fungicidal properties of the skin are attributed to the presence of FFA on its surface [8-10]. The authors of hypotheses on the pathogenesis of acne simplex believe that inflammation of the follicular wall of a sebaceous gland is related to the irritating effect of FFA [3, 7, 11]. It has been established that saturated fatty acids with chains of 10-18 atoms of carbon have comedogenic activity [5].

Because of the high sensitivity and informativeness of gas chromatography, it has gained wide use in the last 10 years in biomedical investigations [1].

Our objective here was to develop a gas chromatography method for examining the FFA of sebum cutaneum.

#### Methods

The subjects took a shower in the evening, but did not shave or wash in the morning. Sebum cutaneum washings were applied 12 h after the shower (from 1000 to 1100 hours). A glass cylinder (17 mm in diameter) was placed on a section of the skin; it was filled with 5 ml mixture of ethyl alcohol and ethyl ether (1:2), kept there for 10 s and decanted into a test tube. This procedure was repeated twice. The combined washings were kept for further tests at the temperature of a freezer, -5°C.



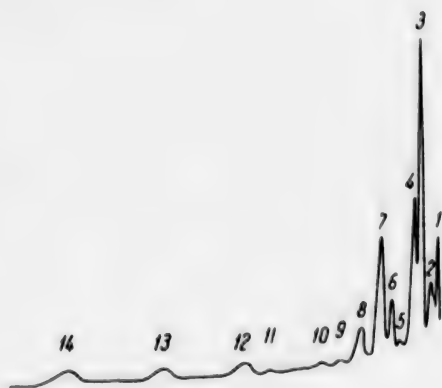


Figure 1.  
Chromatogram of methyl ethers of  
FFA of dorsal skin lipids

Here and in Figure 2:

- 1) myristic (14:0)
- 2) pentadecanoic (15:0)
- 3) palmitic (16:0)
- 4) palmitoleic (16:1)
- 5) (17:0+16:2)
- 6) stearic (18:0)
- 7) oleic (18:1)
- 8) (19:2)
- 9) arachic (20:0)
- 10) (21:0)
- 11) not identified
- 12) behenic (22:0)
- 13) (23:0)
- 14) lignoceric (24:0)

Recovery of methyl ethers of fatty acids was effect in standard solutions of acids using  $\text{BF}_3$ -methanol (14%) reagent of the Fluca firm (FRG). Optimum conditions for etherification were selected on the basis of evaluating fullness of methylation of standard FFA solutions in the presence of bound acids. We obtained a calibration function for quantity of methyl ethers as related to quantity of FFA in the presence of triglycerides, esters of cholesterol and phospholipids. Lipid extract was evaporated from the skin surface (5 ml) in a flow of nitrogen; we added 0.5 ml  $\text{BF}_3$ -methanol reagent (14%) and allowed it to stand for 10 min at room temperature. We then added 0.1 ml zinc acetate (2% solution) and shook the mixture. The obtained methyl ethers were extracted twice with hexane (0.4 ml). The combined batches of extracts were evaporated in nitrogen; we then added 20  $\mu\text{l}$   $\text{CCl}_4$  and placed 4  $\mu\text{l}$  solution in the chromatograph.

We used a Tsvet-100 chromatograph with flame-ionization detector and glass column 4 m long filled with 5% diethylene glycol succinate on gasochrome Q. Velocity of nitrogen carrier gas constituted 50 ml/min, hydrogen 40 ml/min and air 300 ml/min. Operating temperature for column was 100-200°C (programming at 3°C/min) and 180°C.

We examined lipid extracts from the surface of the face, hands and back of healthy men in two age groups (25-35 and 45-55 years).

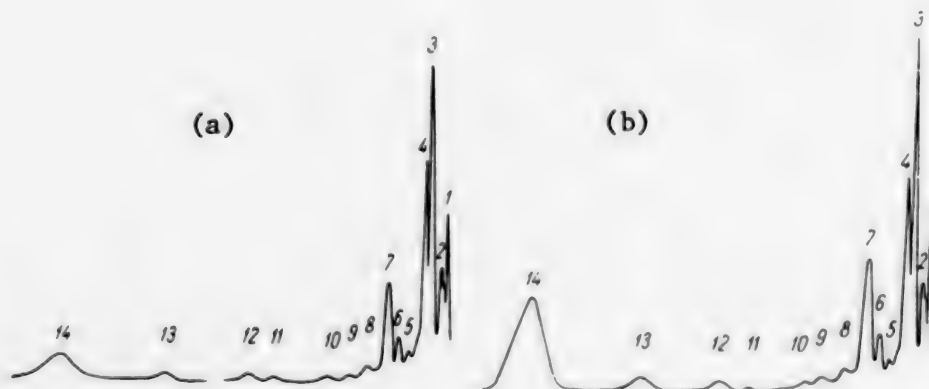


Figure 2. Chromatogram of methyl ethers of lipid FFA on facial skin surface of men 25-35 (a) and 45-55 (b) years old

## Results and Discussion

The studies revealed that higher fatty acids with carbon atom number of 14-24 were the main components of skin surface lipids. Figure 1 illustrates a typical chromatogram of lipid washings from the dorsal skin. Table 1 lists the composition of FFA in lipid washings from the skin of the back and hands of men 45-55 years of age. We failed to demonstrate appreciable differences in composition of sebum FFA between the two skin areas in this age group.

Table 1.

FFA content (%) in lipids on skin of the back and hands of men 45-55 years of age ( $M \pm m$ )

Acid code	Skin surface of	
	back	hand
14:0	4.91 $\pm$ 0.44	3.36 $\pm$ 0.35
15:0	3.87 $\pm$ 0.71	2.99 $\pm$ 0.43
16:0	23.93 $\pm$ 1.53	22.89 $\pm$ 0.76
16:1	10.75 $\pm$ 1.16	9.63 $\pm$ 0.75
16:2 + 17:0	0.79 $\pm$ 0.10	0.35 $\pm$ 0.10
18:0	5.55 $\pm$ 0.44	7.98 $\pm$ 0.66
18:1	14.26 $\pm$ 1.26	17.26 $\pm$ 0.43
18:2 + 19:0	4.16 $\pm$ 0.45	4.79 $\pm$ 0.50
20:0	0.74 $\pm$ 0.13	0.91 $\pm$ 0.28
21:0	0.95 $\pm$ 0.27	0.38 $\pm$ 0.14
Not identified	0.23 $\pm$ 0.16	0.21 $\pm$ 0.21
22:0	3.87 $\pm$ 0.22	4.39 $\pm$ 0.50
23:0	6.61 $\pm$ 1.01	5.43 $\pm$ 0.93
24:0	19.39 $\pm$ 3.08	19.46 $\pm$ 0.97

Table 2.

FFA content (relative %) in lipids on facial skin of men in the two age groups

Acid code	Subjects' age, yrs	
	25-35	45-55
14:0	5.27 $\pm$ 0.97	3.59 $\pm$ 0.18
15:0	8.26 $\pm$ 1.42	5.20 $\pm$ 0.56
16:0	24.19 $\pm$ 1.22	17.83 $\pm$ 0.65
16:1	17.41 $\pm$ 1.62	13.58 $\pm$ 2.30
16:2 + 17:0	1.46 $\pm$ 0.08	0.91 $\pm$ 0.18
18:0	3.75 $\pm$ 0.45	4.09 $\pm$ 1.35
18:1	13.56 $\pm$ 0.70	13.91 $\pm$ 1.31
18:2 + 19:0	3.48 $\pm$ 0.48	4.57 $\pm$ 0.46
20:0	0.34 $\pm$ 0.14	0.95 $\pm$ 0.33
21:0	0.40 $\pm$ 0.12	1.87 $\pm$ 0.87
Not identified	0.19 $\pm$ 0.13	0.57 $\pm$ 0.50
22:0	1.25 $\pm$ 0.42	3.68 $\pm$ 0.56
23:0	2.21 $\pm$ 0.31	5.19 $\pm$ 0.63
24:0	18.17 $\pm$ 4.57	24.06 $\pm$ 3.93

Figure 2 illustrates typical chromatograms of ethyl ethers of FAA in lipid washings from facial skin of men 25-35 and 45-55 years of age. Table 2 lists the composition of FAA in lipid extract from washings from facial skin surface of men in the two age groups. A comparison of these data revealed that there was a higher percentage of biologically valuable  $C_{14}$ - $C_{18}$  acids and a lower percentage of long-chain  $C_{20}$ - $C_{24}$  acids.

Thus, we demonstrated differences in fatty acid composition of lipids in facial sebum in males of different age groups, which requires a differentiated approach to selection of hygienic agents.

In view of the lack of appreciable differences in fatty acid composition of sebum cutaneum on the back and hands, it is recommended that the physiological-hygienic condition of the skin be evaluated in washings from the hand skin surface passed through a "sluice," similarly to how venous blood is drawn for biomedical tests.

The proposed method of gas chromatographic analysis permits evaluation of the composition of the main fraction of skin surface lipids, FFA.

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INVESTIGATION OF MINERAL NUTRITION OF A NEW FORM OF MICROALGAE TO BE USED IN BIOLOGICAL LIFE-SUPPORT SYSTEMS

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[Article by M. A. Levinskikh and O. G. Livanskaya]

[Text] *Closteriopsis acicularis* var. *africana* Hind unicellular green algae attracted the attention of researchers because, unlike *Chlorella*, it has a readily broken down cell membrane and, according to preliminary data, its biomass contains considerable amounts of readily assimilated carbohydrates. It is felt that inclusion of this alga in the photoautotrophic part of a biological life-support system (BLSS) for man could optimize its features considerably.

One of the main questions in studying unicellular algae as applied to human BLSS is their biogenic element requirements. Such knowledge is necessary in order to provide optimum mineral nutrition for algae and high productivity, but mainly to maintain these conditions on a specific level during continuous cultivation in the mode of medium recirculation, which requires constant replenishment of biogenic elements in strict accordance with their removal with the collected harvest [3].

Previously, this form of algae had not been the object of laboratory investigation, and we found no information whatsoever in the literature concerning its mineral nutrition and requirements as to the individual biogenic elements. Our objective was to obtain data on biogenic element requirements of algae during accumulating cultivation under different cultivation conditions.

#### Methods

We conducted experiments under two different cultivation conditions: 1) extensive, in flasks illuminated by fluorescent 10-12 klux lamps with around the clock aeration with a mixture of air and 1-2% CO<sub>2</sub>, and 2) intensive, in a rotation type reactor with up to 60 klux illumination on the surface and aeration with air with CO<sub>2</sub> in a concentration of 2-5%. Algal biogenic element requirements were studied according to removal of elements from the medium during culture growth and their accumulation in biomass. The first stage of these studies, to which this report pertains, was conducted with accumulating cultivation of algae. Removal and accumulation of elements was studied in the linear segment of the growth curve in the presence of all elements of mineral nutrition.

When we started using this form in laboratory cultivation, we proceeded from the fact that it is a representative of chlorococcal algae; consequently, its biogenic element requirements could be similar to those of the well-studied *Chlorella*. For this reason, we first used a nutrient medium that had been previously developed for intensive cultivation of *Chlorella* [2]. Preliminary experiments were conducted with it. They revealed that *Closteriopsis*, just like *Chlorella*, grows well with use of different sources of nitrogen (nitrates, urea and ammonia nitrogen), and they tolerate high concentrations of nitrogen in the medium. The form in question does not require macroquantities of sodium and calcium in the medium. The optimum range of medium acidity for it was found to be similar to the one previously found for *Chlorella*, and it constituted 6-8.

## Results and Discussion

Table 1 lists the amounts of elements removed and accumulated in both modes of cultivation of *Closteriopsis*.

Table 1.

Amounts of mineral nutrients removed from medium and accumulated in biomass (mg/g dry matter) in accumulating cultivation of *Closteriopsis*

Cultivation conditions	Element	Removal from medium	Accumulation in biomass	Utilization, %
Extensive	N	80.2 ± 3.4	79.7 ± 5.4	99.4
	P	15.5 ± 1.0	14.8 ± 1.1	95.5
	S	5.0 ± 0.5	4.5 ± 0.3	90.0
	Mg	6.2 ± 0.2	5.4 ± 0.2	87.1
	K	—	8.2 ± 0.8	—
Intensive	N	42.5 ± 3.2	38.1 ± 0.8	89.7
	P	5.1 ± 0.6	4.9 ± 0.4	96.1
	S	3.8 ± 0.5	3.2 ± 0.2	97.0
	Mg	3.6 ± 0.9	2.9 ± 0.6	80.5
	K	—	5.4 ± 0.3	—

Note: Means and arithmetic mean error are listed from 8 replicas of experiment.

Table 2.

Composition of balanced, corrective solutions for continuous cultivation of *Closteriopsis* (mg/g dry matter)

Mineral salt	Cultivation conditions	
	extensive	intensive
KH <sub>2</sub> PO <sub>4</sub>	68.0	22.0
MgSO <sub>4</sub> ·7H <sub>2</sub> O	63.0	37.0
HNO <sub>3</sub>	360.0	191.0

The data in Table 1 show that in the accumulating mode *Closteriopsis* cell biogenic element requirements depend on the degree of intensification of the cultivation process. With high intensity of light, when algal output reached 10.0 g/ℓ/day, element

requirement diminished, particularly N and P. In biomass grown under intensive conditions, there was 1/2 the N and 1/3 the P in comparison to cells grown under low illumination (culture productivity was about 1 g/ℓ/day). These findings are indicative of high lability of chemical composition of biomass in the form of algae we studied. N uptake by *Closteriopsis* cells, which was 42.5 mg/g dry matter with intensive cultivation, was close to the critical level for *Chlorella*. A decline in nitrogen content of *Chlorella* cells to this level, with nitrogen starvation, leads to total arrest of algal growth. This concentration of N in *Chlamydomonas* cells results in about 50% of nominal rate of biomass increment [1].



A comparison of removal of elements from the medium to their accumulation in biomass demonstrated a difference in the direction of decreased accumulation, as compared to removal. This decrease was minimal for anions and did not exceed 10%, which is indicative of a high degree of utilization by cells of elements absorbed from the medium. This difference is attributable essentially to secondary release into the medium of absorbed elements in the form of metabolic products. The extent of these differences was about the same as for *Chlorella* [2].

We made calculations for corrective solutions to be used in nonflow-through cultivation of *Closteriopsis* on the basis of the obtained data on removal of elements with biomass (Table 2).

Preparation of formulas for corrective solutions enables us to turn to the next stage of investigation of this form of algae, i.e., development of technology for continuous cultivation with recycling of medium.

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